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16. ABSTRACT
Static 96-h LC50 values for 12 water-soluble herbicides with yearling coho salmon in freshwater were: acrolein 68 ug/l; dinoseb 100 ug/l; picloram 5.0-17.5 mg/l; 2,4,5-T (triethylamine) > 10 mg/l; atrazine > 15 mg/l; diquat 30 mg/l; amitrole-T 70 mg/l; paraquat 76 mg/l; dicamba > 100 mg/l; and Krenite and 2,4-D (dimethylamine) > 200 mg/l. Amitrole-T, diquat, and paraquat exposure in freshwater reduced the survival of salmon smolts placed in seawater. Diquat also inhibited downstream migration of smolts. Under normal field use, acrolein and dinoseb could produce mortality of all life stages of salmonids if treated irrigation waters were released into streams prior to herbicide inactivation. The use of diquat at recommended treatment levels could reduce downstream migration of smolts and decrease survival in seawater. All other herbicide formulations tested appeared to have no effect on smolting of coho salmon.

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This report has been reviewed by the Corvallis Environmental Research Laboratory, U. S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Environmental Protection Agency, nor does mention of trade names of commercial products constitute endorsement or recommendation for use.

FOREWORD

Effective regulatory and enforcement actions by the Environmental Protection Agency would be virtually impossible without sound scientific data on pollutants and their impact on environmental stability and human health. Responsibility for building this data base has been assigned to EPA's Office of Research and Development and its 15 major field installations, one of which is in the Corvallis Environmental Research Laboratory (CERL).

The primary mission of the Corvallis Laboratory is research on the effects of environmental pollutants on terrestrial, freshwater, and marine ecosystems; the behavior, effects and control of pollutants in lake systems; and the development of predictive models on the movement of pollutants in the biosphere.

This report describes a potentially adverse effect of pollutants on fish such as salmon which must migrate from freshwater to seawater, and demonstrates that under certain conditions exposure to sublethal levels of pollutants can result in mortality when fish subsequently enter seawater. Laboratory test methods are described which should detect this effect in screening tests and the data obtained in this report should advance knowledge on the effects of pollutants in aquatic ecosystems.

J. C. McCarty
Acting Director, CERL

ABSTRACT

The 96-h LC50 values of several herbicides to yearling coho salmon, Oncorhynchus kisutch, were determined. All 96-h tests were conducted under static conditions at 10°C in freshwater of alkalinity and hardness ranging from 70-83 mg/L and 85-93 mg/L (as $CaCO_3$), respectively. The herbicides acrolein and dinoseb were the most toxic of the 12 water soluble herbicides tested, having 96-h LC50 values of 68 and 100 ug/L, respectively. Atrazine, diquat and picloram were moderately toxic in freshwater with 96-h LC50 values ranging from 10-30 mg/L.

Fish exposed to Amitrole-T, diquat and paraquat in freshwater all exhibited dose-dependent effects in subsequent seawater entry tests. The other herbicides tested produced little or no dose-related mortality when fish were challenged with seawater. No apparent affects on the (Na,K)-stimulated ATPase activity of the gills were observed with any of the herbicides tested.

The effect of sublethal concentrations of Tordon 101, dinoseb and diquat on migratory disposition was tested by releasing herbicide-exposed salmon into a natural stream; only diquat produced a significant reduction in downstream migration.

Under normal (field) application acrolein and dinoseb could affect survival of all life stages of salmonids if water from treated irrigation ditches were released into the stream or river without sufficient holding or detoxifying time. The use of diquat at recommended treatment levels could reduce downstream migration of smolts and possibly affect their survival in seawater. All other herbicide formulations tested appeared to have no effect on smolting of yearling coho salmon; however, atrazine has been shown to affect growth of young salmonids and survival of invertebrates at very low concentrations. Effects of the herbicides on other life stages of coho salmon or different formulations of the herbicides might produce considerably different results.

This report was submitted in fulfillment of grant R-804283 by the Oregon Department of Fish and Wildlife under the partial sponsorship of the U. S. Environmental Protection Agency. This report covers the period January 5, 1977 to June 30, 1978.

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The authors gratefully acknowledge Drs. G. A. Chapman, A. V. Nebeker and H. H. Wagner who provided constructive criticism on the manuscript.

SECTION I

CONCLUSIONS

1. Acrolein and dinoseb were the most toxic of the herbicides tested having 96-h LC50 values of 68 and 100 µg/L, respectively.

2. Atrazine, diquat and picloram were moderately toxic in freshwater with 96-h LC50 values ranging from 10-30 mg/L.

3. Fish exposed to Amitrole-T, diquat, and paraquat in freshwater exhibited dose-dependent effects in subsequent seawater entry tests.

4. Freshwater exposure to sublethal concentrations of diquat (5 mg/L for 144 h) resulted in some deaths when fish were challenged with seawater. Diquat exposures of 0.5-3.0 mg/L for 96 h resulted in reduced downstream migration following release of fish into a natural stream.

5. No apparent effects on the (Na,K)-stimulated ATPase activity of the gills were observed with any of the herbicides tested.

6. The herbicide formulations tested appeared to have no effect on smolting of yearling coho salmon except for the direct toxicity of acrolein and dinoseb, and the effect of diquat on seawater survival and downstream migration. Therefore, application of these formulations at their recommended levels of use (Oregon Weed Control Handbook, 1977) should not affect smolting.

7. Misuse of chemicals has led to direct loss of fish, and this suggests that stricter enforcement of regulations and the possible need for training and refinement in licensing of applicators is required.

8. The data indicate that some bioconcentration from the water occurred in the fish exposed to dinoseb and Esteron; however, it is low compared to pesticides (e.g. DDT).

9. Some of the chemicals tested produced pronounced histopathological effects in exposed fish; the tissue effects were similar to those produced by toxic agents.

SECTION II

RECOMMENDATIONS

1. The techniques and methodology developed in this and prior studies (Lorz and McPherson 1977, Lorz et al. 1978) are recommended for the determination of pollutant effects on anadromous fishes. The Seawater Entry Test (Lorz et al. 1978) is a quick method for determining if pollutants in freshwater could be potentially harmful to the seawater life history phase.

2. Additional research should be conducted to determine if oil-soluble herbicide formulations are detrimental to smolting. Present methodology needs to be reviewed, however, and probably development of new exposure methods and improved analyses for chemicals in the aquatic environment is required.

3. Other life stages (egg, alevin, and fry-fingerling) should be tested for toxicity and sublethal effects (possibly behavioral studies), especially with herbicides that persist in the environment for extended periods.

SECTION III

INTRODUCTION

Herbicides have become a widely used and nearly indispensable tool in reducing the competition of brushy hardwoods in intensive silviculture of Douglas-fir in the Pacific Northwest. As Norris (1971) pointed out, however, it is necessary to know the behavior of these chemicals to allow their safe use, particularly with respect to water contamination.

Herbicides can enter streams through direct application to stream surfaces, in overland flow during periods of intense precipitation or by leaching through the soil profile. The probability of overland flow or leaching of an herbicide to streams depends largely on the persistence and movement characteristics of the chemical, properties of the soil, and the degree to which precipitation infiltrates the soil surface. Leaching is a slow process capable of moving only small quantities of chemical short distances. Overland flow of water (and herbicide) on forest land seldom occurs because the infiltration characteristics of the forest floor and soil greatly exceed rates of precipitation (Norris and Moore 1971). Norris (1967) reported that direct application of chemicals to stream surfaces is the principal mechanism of chemical entry to aquatic systems. This type of contamination can be prevented or minimized through the use of buffer strips and attention to the details of application.

The opportunities for entry of herbicides from agricultural lands to streams are similar to those from the forest except where herbicides are used to control aquatic or streambank vegetation. Many forest and agricultural herbicides are applied in the early spring and summer months when anadromous salmonids are normally migrating downstream. A careful evaluation of the possible toxic hazards of herbicides to aquatic life is therefore necessary to insure that use of these valuable tools does not cause toxic impacts on aquatic organisms.

Evaluation of the probability of occurrence of toxic impacts on aquatic organisms requires a consideration of two factors. One is the inherent toxic properties of the chemical involved, and the second is the probability that the organisms will be exposed to toxic amounts or concentrations of this chemical. The probability of exposure is related to both temporal and spatial relationships between aquatic organisms, treated areas, application of herbicides and the behavior of the chemical in the environment. Behavior of chemicals in the environment includes their movement, persistence, and fate within the aerial, terrestrial, and aquatic portions of the environment. Of primary importance is the mechanism of chemical entry to streams.

Concern regarding the protection of surface waters and aquatic life has prompted numerous evaluations of the effects of chemicals on aquatic invertebrates and fishes. Much of the toxicological research with aquatic biota has, however, been limited to the development of acute toxicity values to measure the effects of the chemicals on these organisms. More recently, chronic exposure of fish and aquatic organisms has received attention because numerous parameters can be evaluated as indices of toxic effects (Arthur 1970, Eaton 1970, Macek et al. 1976a, b, McKim and Benoit 1971, and Mount 1968). The "laboratory fish production index" as defined by Mount and Stephen (1967) reflects toxic effects on reproduction, growth, spawning behavior, egg hatchability and fry survival. A parameter which has received little attention is the effect of chemicals on seaward migration and saltwater adaptation of anadromous species.

The seaward migration of juvenile coho salmon *(Oncorhynchus kisutch)* normally occurs during the spring of their second year of life. They are fully euryhaline several months earlier (Conte et al. 1966, Otto 1971) provided they have achieved a threshold size of 9 cm. The experimental transfer of juvenile salmonids from freshwater to seawater is followed by a transient but marked disturbance of plasma water-electrolyte balance (Conte et al. 1966, Miles and Smith 1968). This osmotic disturbance is caused by the physiological changes necessary to adapt from freshwater osmoregulation (salt retention, water excretion) to seawater osmoregulation (salt excretion, water retention). These disturbances are minimized at the time of normal seaward migration or "parr-smolt transformation" (Wagner 1974a, b).

Zaugg and Wagner (1973) presented data showing that one of the physiological factors correlated with migratory behavior in steelhead trout *(Salmo gairdneri)* was an elevation of (Na,K)-stimulated adenosine triphosphatase (ATPase) activity in the microsomes of gills. This enzyme activity doubled during the parr-smolt transformation of coho salmon and steelhead trout (Zaugg and McLain 1970, 1972, and Zaugg and Wagner 1973). ATPase activity in salmonids increases rapidly during seawater exposure, reaching a maximum after about 30 days, and is thought to be an important factor in maintaining salt (osmotic and ionic) balance in fish (Epstein et al. 1967, Zaugg and McLain 1970). Lorz and McPherson (1977) showed that sublethal levels of copper inhibited the (Na,K)-stimulated ATPase activity. Several workers have reported "in vitro" inhibition of ATPase by chlorinated hydrocarbon insecticides and polychlorinated biphenyls (Campbell et al. 1974, Koch et al. 1972, and Leadem et al. 1974).

Widespread interest in the occurrence and persistence of chlorinated hydrocarbon insecticides, herbicides and heavy metals in surface waters has resulted in several reviews of the effects of these chemicals on aquatic ecosystems (Cope 1966, Eisler 1973, Eisler and Wapner 1975, Johnson 1968, and Mullison 1970). Our earlier research indicated that copper and mixtures of cadmium or zinc with copper were detrimental to smolting in coho salmon (Lorz and McPherson 1977, Lorz et al. 1978). Therefore in the studies reported here, we were interested in determining if some of the herbicides used extensively in forestry and agriculture would have similar effects on smolting.

This report presents data on the effects of selected herbicides on survival, seawater adaptability, (Na,K)-stimulated ATPase levels, and downstream migration of yearling coho salmon following acute and chronic herbicide exposure. The report includes a detailed description of methods, a results and discussion section arranged on a compound by compound basis, and a series of appendices. The results and discussion section includes reviews of pertinent literature relevant to toxicity characteristics, and the environmental behavior of each compound at recommended application rates.[1/]

1/ *Application rates from Oregon Weed Control Handbook 1977 unless otherwise noted.*

SECTION IV

METHODS

SELECTION OF TOXICANTS AND EXPERIMENTS

The Environmental Management Section (EMS) of the Oregon Department of Fish and Wildlife provided a list of approximately 30 herbicides that have caused environmental concern in the past few years. This list was reviewed with Dr. Logan Norris (Pacific Northwest Forest and Range Experiment Station) for herbicides that are currently used in forest management and agriculture and could thus be potential contaminants of aquatic ecosystems. The Forestry Science Laboratory agreed to undertake the analysis of water and fish samples for residues of herbicides chosen for study. Although in most forest spray applications oil-soluble formulations are used, we decided to test water soluble herbicide formulations because they allowed simpler design and clean-up of dosing equipment. Equipment designed for an earlier metal study (Lorz and McPherson 1977) could be used with minimum modification and thus a greater number of chemicals could be tested without the development of a new delivery system for oil-soluble herbicides.

The study was designed to determine the effects of freshwater exposure to several herbicides on the acute toxicity of yearling coho salmon and on the subsequent ability of the salmon to adapt to seawater. Following acute exposure to a given herbicide, (Na,K)-stimulated ATPase activity of the gill was monitored and survival in seawater observed. If deaths occurred during the exposure to a herbicide, histological examination of several tissues was conducted.

Based on acute exposures, three of the thirteen herbicides were chosen for further testing. The effects of Tordon 101, dinoseb, and diquat on growth, seawater tolerance, (Na,K)-stimulated ATPase activity, migratory disposition, histopathology, and tissue accumulation of herbicides in yearling coho salmon following 12-15 days exposure were examined. Our study was limited to the smolt life history stage in the salmon's development. Herbicide levels found to have an effect on yearling coho salmon may be considerably different from those which could affect alevins, fry, or fingerling salmon.

EXPERIMENTAL FISH

Most experiments were conducted on 12-to 17-month-old coho of the 1975 year class[2/]. Fish were hatched and reared from fertilized

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[2/] *Year class refers to year of spawning.*

eggs obtained from the Oregon Department of Fish and Wildlife (ODFW) Fall Creek Salmon Hatchery, Alsea River, Oregon, under conditions similar to those reported by Lorz and McPherson (1977). Steelhead fry *(Salmo gairdneri)* from Big Creek Salmon Hatchery (ODFW) were used in the Esteron tests in addition to yearling coho salmon.

EXPOSURE TO TOXICANTS

Toxicants

Twelve water-soluble and one water-emulsifiable herbicides were tested under static exposure conditions. The herbicide formulation tested, its manufacturer, chemical name, and summary of registered uses are listed in Table 1.

Static exposure tests

The static toxicity tests were carried out in 0.61-m diameter fiberglass tanks. Water was continuously aerated and 85% of the 120 L was exchanged once per day. The fish were generally placed in the test tanks 3 days prior to toxicant exposure for acclimatization and recovery from handling. Toxicant solutions were mixed in a separate container prior to introduction into the tanks. The daily exchange of toxicants always started with the control tanks and went to successively higher toxicant concentrations; the mixing container was rinsed following each concentration change. Upon completion of the daily toxicant changes, the mixing bucket was rinsed several times and then flushed overnight with running freshwater. Where possible, toxicant concentrations tested were selected from published LC50 data for the particular herbicide. A minimum of seven replicated concentrations, with 10 fish per test tank, were used for each static toxicity test.

Flow-through tests

Four herbicides (Tordon 101, dinoseb, diquat and Esteron Brush Killer) were used in a flowing water system. This system consisted of a gravity flow diluter (Figs. 1 and 2) capable of delivering 12 L/min to each of 10 exposure tanks (five duplicated concentrations). A volume of 1000 L was maintained in each of the ten 1.54-m diameter fiberglass exposure tanks, and 95% of the water was replaced every 3.7 h. Submersible pumps were used in each tank to provide additional current, aeration and mixing. Yearling coho salmon (210-225/tank, except the Esteron study which utilized 50 fish/tank) were fin-clipped and allowed to acclimate at least one week before the toxicant exposure began. The concentrations were sublethal and based on prior static bioassays. Water and toxicant flows in the diluter were checked at least once daily; only occasional minor adjustments were required.

TABLE 1. HERBICIDES TESTED FOR ACUTE TOXICITY TO YEARLING COHO SALMON[a/].

Common or trade name	Chemical name	Manufacturer	Registered use(s)[b/]
Acrolein	acrolein or acrylaldehyde	Shell Oil Co.-Texas[c/]	Control of submerged aquatic weeds in flowing water.
Amitrole-T(Cytrol^{R})	3-amino-1,2,4-triazole and ammonium thiocyanate	American Cyanamid Co.	Non-crop uses such as right-of-way, industrial premises and ditchbanks.
Atrazine (AAtrex^{R})	2-chloro-4 ethylamino-6-isopropylamino-s-triazine	Ciba-Geigy	Corn, sorghum, perennial ryegrass and winter wheat.
Dicamba (Banvel^{R})	3,6-dichloro-o-anisic acid	Velsicol Chem. Co.	Barley, corn, oats, wheat and pasture and rangeland.
Dinoseb (Premerge 3)	2-sec-butyl-4,6-dinitrophenol	Dow Chemical[d/]	Both non-crop and food-crop uses. Food crops include: alfalfa, cereal grains, fruits, nuts and vegetables.
Diquat (Ortho^{R} Diquat Dibromide)	6,7-dihydrodipyrido (1,2-a:2',1'-c) pyrazinediium ion	Standard Oil (Ortho Div.)	Non-food use; seed crops of alfalfa, clover and vetch plus canals, lakes and ponds.
Esteron^{R} Brush Killer	2,4-D propylene glycol butyl ether ester + 2,4,5-T propylene glycol butyl ether ester	Dow Chemical[e/]	Pasture and rangeland, forest and non-crop uses.
Krennite	Ammonium ethyl carbamoylphosphonate	E.I. du Pont[f/] de Nemours	Brush control on non-cropland areas.
Paraquat-CL	1,1'-dimethyl-4,4'-bipyridinium ion	Standard Oil (Ortho Div.)	Preplant or directed spray on both non-crop and food crops such as alfalfa, fruits, nuts and vegetables.
2,4-D (Amine D)	2,4-dichlorophenoxyacetic acid (dimethylamine formulation)	Diamond Shamrock Chem. Co.	Range and pasture grasses, vegetables, fruit, grains, berries and certain aquatic sites.
2,4,5-T ("Weedar")	2,4,5-trichlorophenoxyacetic acid (triethylamine formulation)	Amchem. Prod. Inc.	On an extended basis for use on pasture and rangeland, forests and non-crop uses.
Tordon^{R} 101	4-amino-3,5,6-trichloropicolinic acid + 2,4-dichlorophenoxyacetic acid both as the trisopropanolamine salts	Dow Chemical Co.	Pasture and rangeland
Tordon 22K (Picloram)	4-amino-3,5,6-trichloropicolinic acid (as potassium salt)	Dow Chemical Co.	Pasture and rangeland

[a/] *Commerical formulations of herbicides purchased from Wilbur Ellis Co. Portland, Oregon unless otherwise noted.*
[b/] *Oregon Weed Control Handbook, 1977.*
[c/] *One liter sample provided by Shell Oil Company, Houston, Texas.*
[d/] *Dow Chemical Company supplied 5 gal. Dow Premerge 3.*
[e/] *Oregon State Dept. of Forestry (Astoria) provided 1 gal of Esteron Brush Killer.*
[f/] *Dr. M. Newton, OSU School of Forestry, provided sample.*

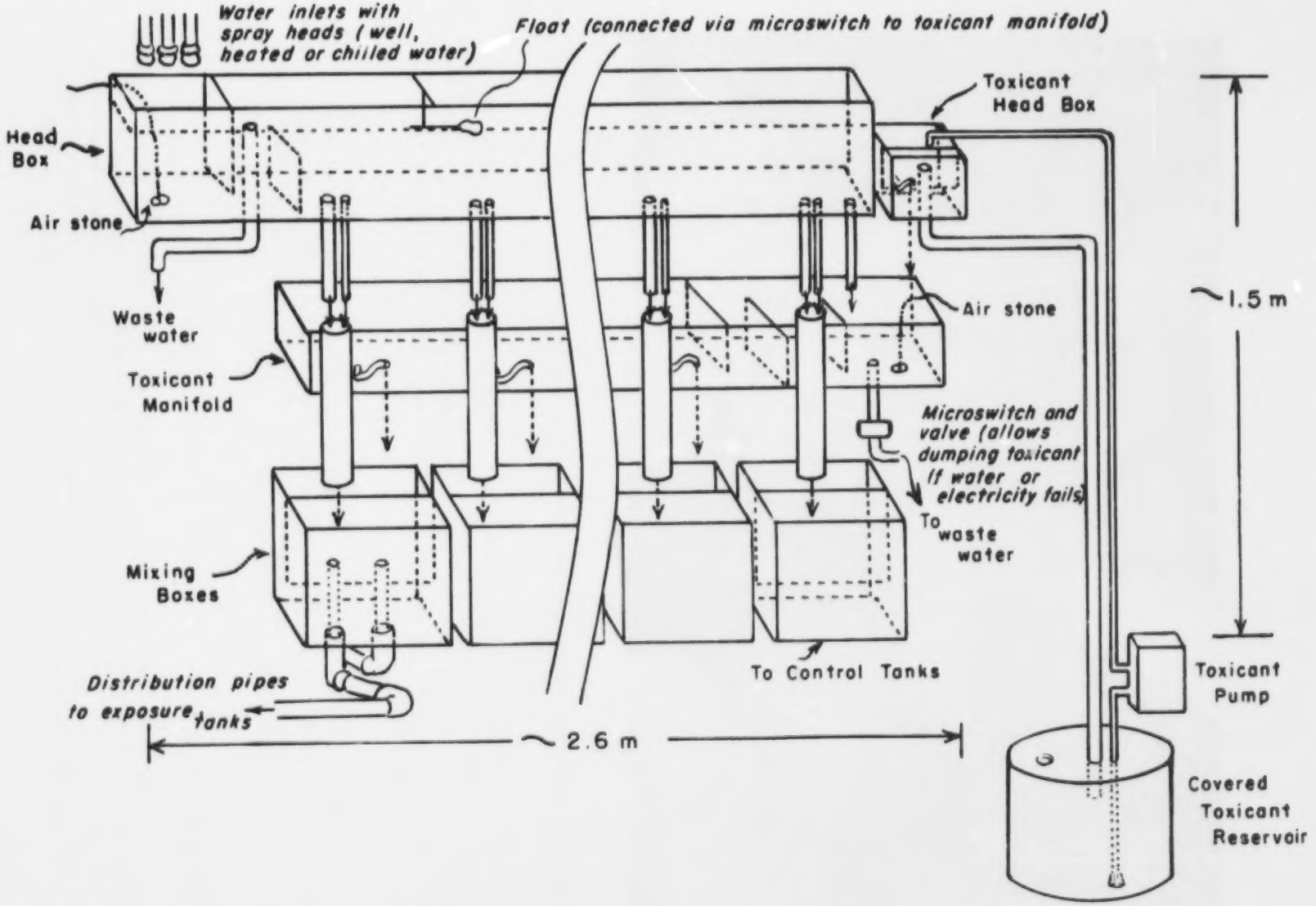

Figure 1. Diagram of flow-through diluter.

Figure 2. Exposure tanks with diluter in background.

In addition, smaller groups of fish (about 50/tank) were exposed to flowing toxicant in 0.61-m fiberglass tanks. Flows (5 L/min, 95% replacement every 1.5 h) to the 120 L tanks were provided by a siphon from mid-depth of the 1.54-m tanks used for chronic exposure. All tanks were covered to prevent loss of fish.

An activated carbon filter (Marking and Piper 1976) was set up to remove herbicides from the effluent waters prior to discharge from the laboratory. The carbon filter was changed every 8 days and incinerated. This is considered one of the better methods for destruction of small quantities of waste pesticide chemicals (Kennedy et al. 1969).

WATER QUALITY

Water for the study came from several wells. The chemical characteristics of the water have been monitored during the past years (Lorz and McPherson 1977). Alkalinity, hardness, dissolved oxygen, pH and ammonia in the static exposure tanks were measured routinely at the laboratory (Table 2). In the flow-through system the dissolved oxygen was always >8.5 mg/L,

pH @7.03, ammonia $\leq$0.10 mg/L and hardness @100 mg/L as $CaCO_3$. Water temperature was maintained at 10 $\pm$ 1°C in all static tests. In the flow-through test the temperature pattern was similar to the average of the semi-monthly maximal and minimal temperatures of the North Fork of the Alsea River (9.0°C in March increasing to 12.3°C in June). Water temperatures were monitored in the static and flow-through systems with continuous recording thermometers.

CHEMICAL ANALYSIS

The concentration of each herbicide was analyzed from composite water samples for each exposure tank. However, no analyses, were conducted for acrolein or krenite. In the static toxicity tests a composite water sample was obtained by combining daily samples of 80 ml of test water in a container with an appropriate fixative. The water samples were generally taken several hours after the daily exchange. In the flow-through system a weekly composite water sample was obtained by combining daily samples of 50 ml of test water. At least two weeks usually elapsed between sampling and subsequent extraction and analysis. During this period the samples were stored in a cool area.

The analytical method for each herbicide tested is described in Appendix 1. In a number of tests the measured concentrations varied considerably from the desired or nominal; however, no explanations are known for the deviations. The discrepancies (between measured and nominal concentration) possibly resulted because of problems in extraction or analysis or may have been due to the physical-chemical behavior of the herbicide solution in the exposure tank.

GILL ATPASE ACTIVITY

(Na,K)-stimulated ATPase activity was measured on individual fish by the whole gill homogenate method (Johnson et al. 1977). Previous characterization of this enzyme assay was conducted on coho salmon (Lorz et al. 1978). Enzyme activity was measured at 37°C. The released inorganic phosphate was measured by the method of Ernster et al. (1950). (Na,K)-stimulated ATPase activity was calculated as the difference between rates of inorganic phosphate liberated in the presence or absence of 0.5 mM ouabain. Protein was measured by a modification of the method of Lowry et al. (1951). Sample sizes ranged from 5-6 fish in static tests and 10-20 fish in the flow-through system.

TOLERANCE TO SEAWATER

Generally groups of 10-20 fish from each exposure tank (following 144-h static, 144-h flow-through, or 15-day flow-through toxicant exposures) were tested for tolerance to seawater. The percentage of these yearling coho salmon surviving the seawater challenge thus provided a presumptive measure of the fish's osmoregulatory ability. Exposed fish and controls were transferred directly from the exposure tanks into 0.61-m fiberglass tanks containing 120 L of natural seawater. The seawater was continuously aerated and 85% of the 120 L was exchanged once daily.

TABLE 2. CHEMICAL CHARACTERISTICS OF TEST WATER DURING STATIC TOXICANT EXPOSURE, AVERAGE VALUE WITH RANGE IN PARENTHESES.

Toxicant	Dissolved oxygen (mg/L)	Ammonia (mg/L NH_3-N)	pH[a]	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)	Number of analyses
Amitrole-T	10.5 (9.2-11.0)	[b]	7.5-7.6	79 (75-82)	100 (100-101)	4
Krennite	10.8 (10.7-11.0)	[b]	7.5-7.6	79 (76-80)	100 (99.5-100.5)	4
Dicamba	10.7 (10.2-10.9)	0.32 (0.22-0.42)	7.7-7.8	83 (82-84)	102 (101-102)	8
Dinoseb	11.0 (10.7-11.2)	0.52 (0.25-0.75)	7.7-7.8	81 (77-83)	101	4
Tordon ^{R}101	10.0 (9.0-10.8)	0.67 (0.65-0.72)	7.4-7.6	81 (30-83)	102	10
Tordon 22K (Picloram)	10.5 (8.6-11.2)	0.32 (0.25-0.41)	[b]	[b]	[b]	12
Atrazine	11.1 (11.0-11.4)	0.29 (0.22-0.42)	7.4-7.6	84	101 (100-101)	8
2,4-D	10.7 (10.4-10.8)	0.45 (0.40-0.48)	7.2-7.6	[b]	105	4
2,4,5-T	11.0 (11.0-11.1)	(0.5-<0.7)	7.5	77 (76-81)	99	4
Esteron^{R} Brush Killer	10.6 (10.2-10.8)	[b]	7.4	80	100	1

[a] *pH value taken after 2 min of gentle stirring.*
[b] *No data collected.*

Salinity of the water was maintained at 30 ± 0.5 °/oo with a temperature of 10 ± 1°C. The seawater exposure period was a minimum of 10 days; if mortality was still occurring, longer observation periods were used.

ASSESSMENT OF COEFFICIENT OF CONDITION

Each month, after a 24-h starvation period, about 40 fish were selected randomly from the stock and exposure tanks, anesthetized, weighed, and measured. Individual fish were weighed to 0.1 g and fork length was determined to 0.1 cm. The coefficient of condition (K) was determined for each fish in the sample using the formula $K = 100\ W/L^3$, where W denotes weight in grams and L denotes fork length in centimeters (Hoar 1939). Any change in condition factor compared to controls probably reflected an effect of the herbicide on metabolic processes because the feeding behavior appeared to be unaffected except in those fish exposed to dinoseb.

HISTOLOGICAL EXAMINATION

Gill, liver and kidney tissue were usually excised from freshly killed fish and preserved in Bouins solution for histological examination. Three to five fish were collected from herbicide concentrations that caused death during the static exposure. Five chronically exposed fish were collected from each Tordon 101, dinoseb and diquat concentration. Dr. J. D. Hendricks, Dept. of Food Science and Technology (Oregon State University) made the histological examination of the fish. Dr. Hendrick's analyses are in Appendix II, but will be referred to in the results and discussion whenever histopathological damage was noted for a particular herbicide.

DOWNSTREAM MIGRATION

The effect of Tordon 101, dinoseb and diquat on migratory disposition was assessed by releasing marked control and herbicide-exposed yearling coho salmon into a tributary of the North Fork Alsea River and trapping them 6.4 km downstream. The trap was checked daily for the first 10 days following release, then every second or third day thereafter. On the day prior to release, about 100 fish from each 1.54-m exposure tank (long exposure) and 50 fish from each 0.61-m tank (short exposure) were anesthetized in MS-222, weighed and marked by freeze branding and a fin clip. No fish smaller than 11 cm were released as the "parr-smolt transformation" is markedly size dependent. Releases were made between April 13 and May 26, 1977, the normal time of seaward migration of wild juveniles. Trapping was terminated July 6, 1977, more than a month after the last release.

Probability estimates for downstream migration occurring from each release were tested for significance between the control group and each treatment group. Significance (P=0.05) was determined by the z test where the null hypothesis tested was:

H_0: $P_T = P_C$

H_A: $P_T \neq P_C$

$$z = \frac{P_C - P_T}{\sqrt{\frac{P_C \cdot (1-P_C)}{n_C} + \frac{P_C \cdot (1-P_C)}{n_T}}}$$

and where: $P = a/n$

a = number of migrants
n = number of fish released.

A 7-km section of the stream was electrofished from above the release sites to the weir in an attempt to collect non-migrants. No coho that had been released earlier were found, although native cutthroat and rainbow of comparable size to the released fish were caught. There were only two small areas in the stream where our electrofishing gear appeared inadequate because of the depth of the water. Thus, fish that had not migrated by early July apparently died as a result of predators, stress, natural causes or latent effects of the toxicant exposure.

SECTION V

RESULTS AND DISCUSSION

From November 1976 to June 1977 we completed static toxicity tests with 13 herbicides. These tests included determinations of acute lethality, (Na,K)-stimulated ATPase activity of the gills, and tolerance to seawater following exposure to herbicides in freshwater. In addition, tolerance to seawater, (Na,K)-stimulated ATPase activity, and migratory disposition were investigated using yearling coho salmon previously exposed to Tordon 101, dinoseb and diquat in a flowing freshwater toxicant system. The results of tests with each herbicide are discussed below.

ACROLEIN

Review of Literature: Toxicity to Fish and Behavior in the Environment

Acrolein is the common name for acrylaldehyde or 2-propenal, and is manufactured under the trade name of Aqualin^{R}. It is registered for use in flowing water only (rivers, canals, and irrigation ditches) for the control of submerged and floating aquatic weeds. Acrolein has recently found use as a contact aquatic herbicide and is also used as a molluscicide for control of river-dwelling snails (Tweedy and Houseworth 1976).

Acrolein is reported to be hydrolyzable and volatile. It polymerizes slowly in the presence of air, producing an insoluble white precipitate. Macek et al. (1976a) referring to some unpublished work by Battelle (1970) noted acrolein's extreme volatility. When acrolein was applied at 0.7 mg/L in an irrigation ditch flowing at approximately 130 cfs, its concentration decreased by 98% over a 19-mile section at a temperature of 17.8°C (64°F), while at 8.9°C (48°F) only 62% was lost in a 27-mile stretch. Moen (1961) found that acrolein quickly disappeared from a pond when it was applied at 0.5 mg/L.

Acrolein is very toxic to fish (Table 3). Bond et al. (1960) reported 24-h LC50's of 80 µg/L and 65 µg/L acrolein for chinook salmon *(Oncorhynchus tshawytscha)* and rainbow trout *(Salmo gairdneri)*, respectively, under static conditions. These findings were similar to 24-h LC50 values of 75 µg/L for fingerling brown trout *(Salmo trutta fario)* and 46 µg/L acrolein for bluegills *(Lepomis macrochirus)* as reported by Burdick et al. (1964) using a continuous flow-through system. Folmar (1976) reported the 96-h LC50 for rainbow trout to be 140 µg/L acrolein.

TABLE 3. ACUTE TOXICITY OF ACROLEIN TO VARIOUS FISH SPECIES.

Species	Toxicity test	LC 50[1] (µg/L)	pH	Temperature	Alkalinity	Hardness	Reference
Amia calva (bowfin)	static, lab	62 (24 h)	7.2-7.3	69-72°F	89-93	40-41	Louder and McCoy (1962)
Carassius auratus (goldfish)	field pond	2000(24 h)					Jordan et al. (1962)
Carassius auratus	static, lab field tests	1000(3 h)[2] 2000(24 h)[2]		69-74oF			St. Amant et al. (1964)
Fundulus similis (killifish)	flowing, lab	240 (48 h)[3]					Butler (1965)
Gambusia affinis (mosquitofish)	static, lab	149 (24 h) 61 (48 h)	7.1-7.3	69-72°F	89-93	40-41	Louder and McCoy (1962)
Lepomis macrochirus	flowing, lab	77 (24 h)[4]		60°F			Burdick et al. (1964)
Lepomis macrochirus	field pond	2000(24 h)					Jordan et al. (1962)
Lepomis macrochirus (bluegills)	static, lab	140 (24 h) 100 (96 h)	7.2-7.3	69-72°F	89-93	40-41	Louder and McCoy (1962)
Micropterus salmoides (largemouth bass)	static, lab	183 (24 h) 160 (96 h)	7.2-7.3	69-72°F	89-93	40-41	Louder and McCoy (1962)
Oncorhynchus tshawytscha (chinook salmon)	static, lab	80 (24 h) 1350(2 h)[2]	7.4-7.7	20°C	41-71		Bond et al. (1960)
O. kisutch (coho salmon)	static, lab	68 (96 h)	7.4-7.6	10°C	79-82	100-101	Lorz et al. (This study)
Pimephales promelas (fathead minnow)	static, lab	150 (24 h) 115 (48 h)	7.2-7.3	69-72°F	89-93	40-41	Louder and McCoy (1962)
Pimephales promelas	flowing, lab	84 (144 h)	6.6-6.8	25°C	30	32	Macek et al. (1976a)
Rasbora heteromorpha (harlequin fish)	flowing, lab	140 (24 h) 60 (48 h)		20°C		20	Alabaster (1969)
Salmo gairdneri (rainbow trout)	static, lab	5000(24)NE[5]	7.5-8.2	55°F			Applegate et al. (1957)
	flowing, lab	140(24)[6]					Folmar (1976)
	static, lab	65 (24)	7.4-7.7	20°C	41-71		Bond et al. (1960)
Salmo trutta fario (brown trout)	flowing, lab	46 (24)		60°F			Burdick et al. (1964)

[1] Concentration of acrolein causing 50% mortality of exposed fish in time given (except as noted).
[2] Total mortality in time given.
[3] Test carried out in flowing seawater.
[4] 40% mortality.
[5] No toxic effect of concentration tested; (no information as to percent active ingredient or why compound reacted so differently).
[6] Unpublished data fish pesticide lab. Columbia Mo.

Fish exhibited a narrow range of susceptibility to acrolein. Louder and McCoy (1962) noted the 24-h and 96-h LC50 values for largemouth bass *(Micropterus salmoides)*, bluegills, bowfins *(Amia calva)*, mosquito fish *(Gambusia affinis)* and fathead minnows *(Pimephales promelas* Rafinesque) ranged from 62 to 183 µg/L. Burdick et al. (1964) found reduced toxicity corresponding to increasing size for bluegills but not for brown trout fingerlings.

Macek et al. (1976a) investigated the effect of chronic exposure to acrolein on fathead minnows. These authors noted that adult fathead minnows exposed to acrolein concentrations of 0 to 41.7 µg/L for 30 to 60 days showed similar rates of spawning, growth, and survival. However, they observed only 2% survival of larval fish exposed to 41.7 µg/L acrolein. The 6-day incipient LC50 for fathead minnows exposed to acrolein was 84 µg/L, and the estimated maximum acceptable toxicant concentration (MATC) was >11.4 <41.7 µg/L.

Under field conditions, Green (1960) found that acrolein applied at 1.0 to 2.0 mg/L could kill carp, *Cyprinus carpio*, and threadfin shad, *Dorosoma petenense*. Largemouth bass and bluegill appeared not to be harmed by 5.0 mg/L Aqualin, but Moen (1961) found Aqualin to be toxic to fish at 0.5 mg/L when applied to a pond. Meyer (1961) found Aqualin was toxic to fish when applied to ponds at rates as low as 0.2 mg/L. Catfish, *Ictalurus* sp. and sunfish, *Lepomis* sp. succumbed at 1 to 2 mg/L Aqualin, while buffalo fish, *Ictiobus* sp. were killed at 0.2 mg/L. Chemical control of filamentous green algae was investigated by Jordan et al. (1962), but they found acrolein to be erratic in its performance and the only herbicide tested that injured fish. Furthermore, they noted 50% mortality of bluegill and goldfish in 24 h following the third and fourth applications of acrolein at 2 mg/L. Applications of 4 mg/L produced 98% mortality of the bluegill and 95% mortality of the goldfish within 24 h. Placement of fish in ponds 2 weeks after application of 4 mg/L resulted in death of all of the goldfish and only 3% survival of the bluegills. These findings are contrary to those of Moen (1961), but probable differences in water quality and an eightfold greater Aqualin concentration may account for the extended toxic condition.

During the 1960's there was interest in acrolein as a fish repellent. Louder and McCoy (1962) reported that acrolein had been used successfully by the Iowa State Conservation Commission to drive fish downstream into a weir in the Raccoon River. They concluded, however, that the herbicide was not suitable for collecting fishes in lotic waters since it required a lethal dose to repel fish. Testing of overt avoidance reaction of rainbow trout fry to acrolein revealed that the fry probably would not avoid a lethal concentration (Folmar 1976).

Experimental Results

The 96-h LC50 for yearling coho salmon was estimated at 68 µg/L acrolein (Fig. 3, Table 4). It was the most toxic water soluble herbicide tested. There was no apparent effect of acrolein on (Na,K)-stimulated

ATPase activity of the gills and little effect on seawater tolerance following toxicant exposure (Table 4). Our 96-h LC50 value is similar to values given by Bond et al. (1960) and Burdick et al. (1964) for salmonids. Histological examination of fish tissues (gill, kidney and liver) indicated that acrolein had detrimental effects which appeared to be concentration dependent (Appendix II).

TABLE 4. SURVIVAL AND GILL ATPASE ACTIVITY OF YEARLING COHO SALMON EXPOSED TO ACROLEIN IN FRESHWATER AND THE SUBSEQUENT SURVIVAL FOLLOWING TRANSFER TO SEAWATER (MAY 10-27, 1977).

Concentration[a] (µg/L) nominal	Percent survival[b] (144-h exposure FW)	Gill ATPase[c]	Percent survival (280-h SW)
Control	100	4.9	93.8
5	100	NT[d]	100.0
10	100	4.7	100.0
20	100	NT	100.0
30	100	NT	100.0
50	95	5.2	86.7
75	0	NT	NT[d]
100	0	NT	NT[d]

[a] *Actual concentration not measured.*
[b] *Twenty fish exposed per concentration.*
[c] *Na,K-activated ATPase activity of the gill; µmoles ATP hydrolyzed/mg protein/h, mean of 4 fish.*
[d] *Not measured, no survivors in the higher concentrations.*

The toxicity of acrolein was recently demonstrated on the Rogue River above Grants Pass, Oregon, where Magnicide H, a gaseous form of acrolein, was introduced into an irrigation canal for the control of algae and submerged vegetation (Oregon Department of Fish and Wildlife, notes in Environmental Management Section file August 1977). The release of the treated irrigation water within 24 h of treatment rather than after the recommended holding time of 6 days (manufacturer recommendation on toxicant container) appeared to be the cause of total mortality of fish in a 10-mile section of the Rogue River below the spill. The Oregon Department of Fish and Wildlife estimated that 238,000 fish were killed, including 42,000 salmonids, with an estimated value of $284,000.

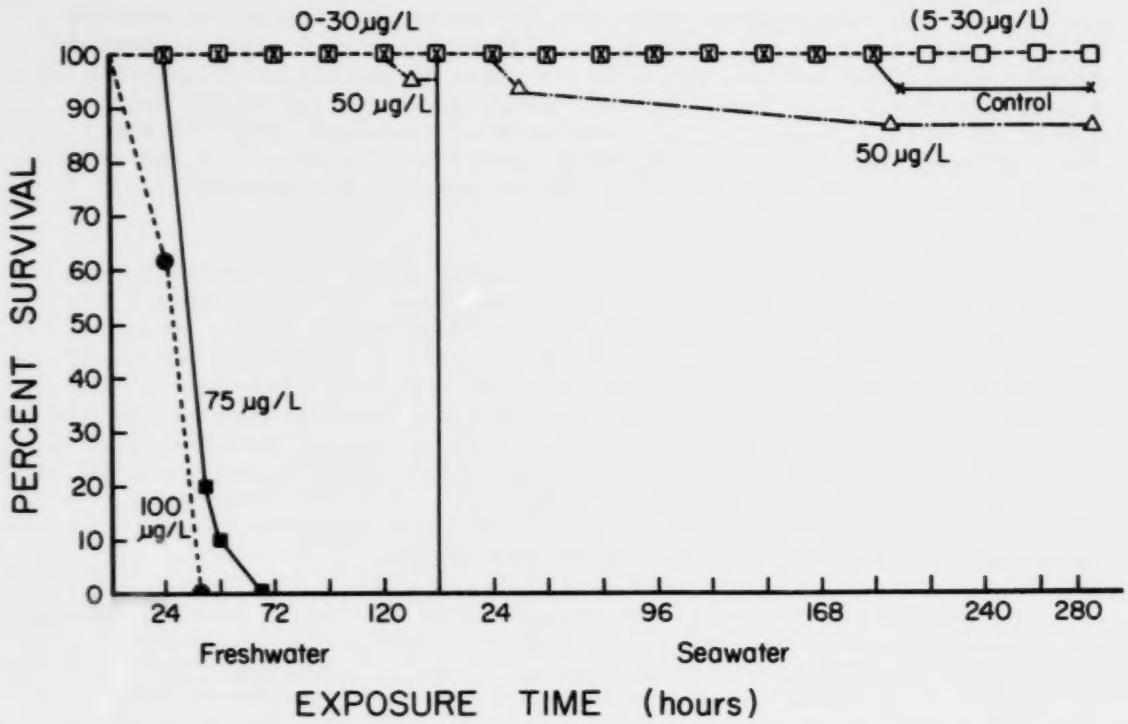

Figure 3. Percent survival of yearling coho salmon during exposure to acrolein in freshwater and subsequent survival upon transfer to seawater.

AMITROLE-T

Review of Literature: Toxicity to Fish and Behavior in the Environment

Amitrole (3-amino-s-triazole) with an equal molar amount of ammonium thiocyanate is formulated under the trade name Amitrole-T. It is registered for non-crop land clearing for right-of-ways, industrial premises, lawn renovation, hardwood nurseries, and ditch banks. Amitrole-T has also been found to be very effective in the control of aquatic vegetation such as water-hyacinths. For spot treatment, Amitrole-T is normally applied at 1/2 lb active ingredient (a.i.) per 12 gal of water. As an area spray it is applied at rates up to 4 lb per acre.

The literature contains many publications describing the fate of applied amitrole. In a study of 55 different California soils, Day et al. (1961) reported that amitrole disappeared rapidly within 2 weeks after application. Rapid decomposition of amitrole was also observed in a similar study in Oregon soils (Freed and Furtick 1961). Sund (1956) noted that amitrole adsorbs rapidly and tightly to soil particles having a high base exchange capacity and a high organic matter content. Amitrole complexed readily with metals in soil.

Riepma (1962) found that soil microorganisms decomposed amitrole rapidly and that the decomposition rate increased with increasing amounts of soil organic matter. Grzenda et al. (1966) observed a tight adsorption of amitrole to the hydrosol and a fairly rapid decomposition of amitrole in a pond. Norris (1970) found that the common brush control herbicides, 2,4-D, amitrole, 2,4,5-T, and picloram were all degraded in the forest floor although the rates of degradation varied considerably. In red alder *(Alnus rubra)* forest floor material, 80% of amitrole was degraded in 35 days.

Amitrole's persistence in water, following aerial application to forest areas for brush control in Oregon, has been studied in several investigations (Marston et al. 1968, Norris 1967, Norris et al. 1966, and Tarrant and Norris 1967). In a study of a stream in a coastal, municipal watershed sprayed with 2 lb/acre amitrole for control of salmonberry, the maximum concentration of herbicide, 155 ppb (in water), was observed 30 min after application (Marston et al. 1968). Only 26 ppb was detected after 2 h and none after 6 days. Norris (1967) monitored amitrole concentration in a stream at a spray site treated at 2 lbs/acre. Five min after spray, 422 µg/L amitrole was detected; 10 hours later the concentration had decreased to 4 µg/L and none was found after 3 days.

The toxicity of Amitrole-T is thought to be similar to that of ammonium thiocyanate (House et al. 1967). Russian studies have indicated that ammonium thiocyanate is lethal to fish at 200 mg/L (Demyanenko 1941). Amitrole-T is believed to be relatively non-toxic to fish (Meyer 1966). Two-inch bluegills tolerated 10 mg/L Amitrole-T for 100 h (U. S. Fish and Wildlife Service 1963). Sanders (1970) noted bluegills survived concentrations of >100 mg/L Amitrole-T for 48 h.

The literature contains a number of publications on the toxicity of amitrole formulated without ammonium thiocyanate. Bond et al. (1960) found the 48-h LC50 for coho salmon to be 325 mg/L amitrole under constant flow conditions and water pH 7.5 to 7.7 and 41 to 71 mg/L total alkalinity. Largemouth bass were able to survive 1,000 mg/L amitrole under static conditions, but in flow-through apparatus all test fish died at this concentration in 6 days. A 24-h LC50 of 1,200 mg/L amitrole was established for bluegill sunfish by Hughes and Davis (1962a) in water of 29 mg/L hardness and pH 6.9.

Hiltibran (1967) investigated the effects of selected herbicides on fish reproduction. Survival of fertilized eggs and fry of bluegill, green sunfish *(Lepomis cyanellus)*, smallmouth bass *(Micropterus dolomieu)* and lake chub sucker *(Erimyzon sucetta)* were not affected by 50 mg/L amitrole under static conditions.

Experimental Results

The 96-h LC50 of Amitrole-T was approximately 70 mg/L for yearling coho salmon (Table 5). There was no apparent effect of the herbicide on the (Na,K)-stimulated ATPase activity of the gills, however, upon transfer to seawater there appeared to be a dose-dependent mortality effect (Fig. 4, Table 5). Our 96-h LC50 value is one-third of that reported in the Russian

studies for ammonium thiocyanate (Demyanenko 1941). The reason for the discrepancy is unknown although differences in water quality or species tested are known to be important variables. Histological examination of dying fish showed degenerative changes occurring in the liver, kidney and gill of fish exposed to 200 mg/L (Appendix II). The deaths observed in seawater generally occurred within the first 24-48 h following transfer from the toxicant and probably were not directly related to impaired osmoregulatory ability.

TABLE 5. SURVIVAL AND GILL ATPASE OF YEARLING COHO SALMON EXPOSED TO AMITROLE-T IN FRESHWATER AND THE SUBSEQUENT SURVIVAL FOLLOWING TRANSFER TO SEAWATER (JAN. 17-FEB. 6, 1977).

Concentration (mg/L)		Percent survival[a]	Gill ATPase[b]	Percent survival
nominal	measured	(144-h exposure FW)		(336 h SW)
Control	0.03-0.06	100	2.1	100
0.25	0.20	100	2.1	100
0.50	0.37	100	NT[c]	100
1.0	0.89-0.97	100	2.7	100
25.0	24.8	100	2.9	56.3
50.0	48.6	55	2.4[d]	12.5
100.0	104.5	14.6	NT	0
200.0	200.7	0	NT	NT

[a] *Twenty to 21 fish exposed per concentration.*
[b] *(Na,K)-stimulated ATPase activity of the gill; mean of 5 fish.*
[c] *Not measured.*
[d] *Mean of 3 fish.*

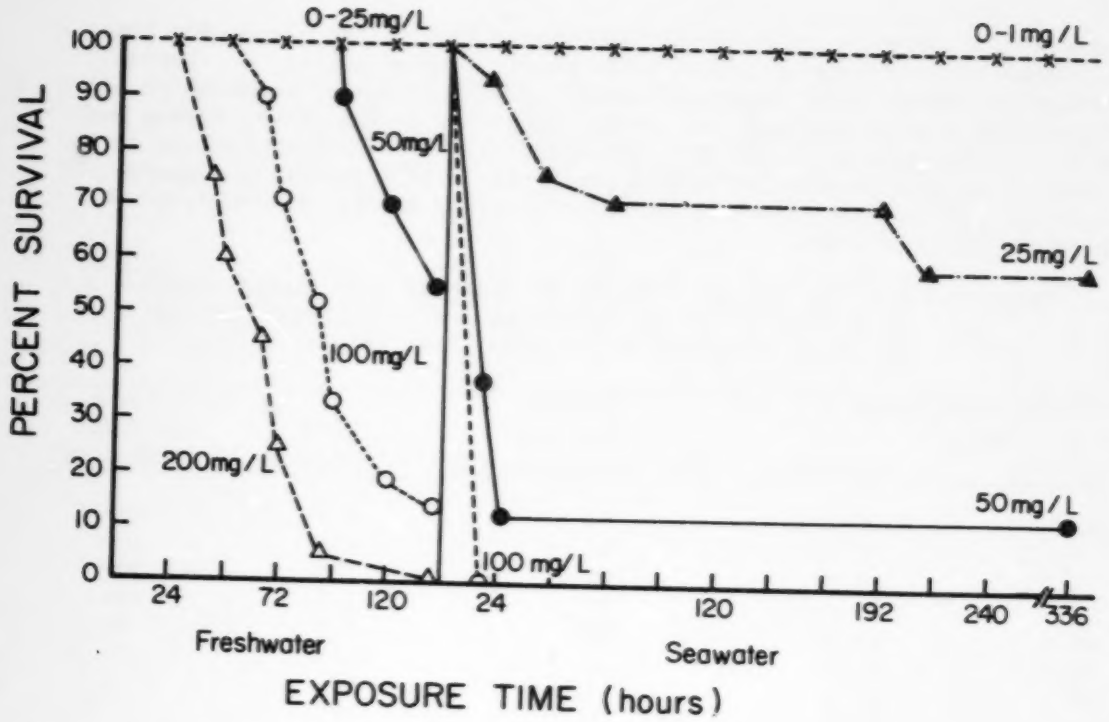

Figure 4. Percent survival of yearling coho salmon during exposure to Amitrole-T in freshwater and subsequent survival upon transfer to seawater.

ATRAZINE

Review of Literature: Toxicity to Fish and Behavior in the Environment

Atrazine (2 chloro-4 ethylamino-6-isopropylamino-s-triazine) is a wide spectrum symmetrical triazine herbicide. It is widely used to control many broad leaf and grass weeds in the production of corn, macadamia nuts, pineapples, perennial ryegrass, sorghum and winter wheat. Atrazine is also employed in some areas for selective weed control in Christmas tree farming, grass-seed production, highway right-of-way clearance, and conifer reforestation. Hall et al. (1972) noted that more than 100 million pounds of atrazine is applied annually to agricultural lands in the United States.

Atrazine is not as strongly bound to soil particles as is the triazine herbicide simazine. Its lower solubility and reduced adsorption add to its mobility in the soil. Axe et al. (1969) studied the residual life of atrazine in the soil and found that only 33% of the herbicide remained after 5 days. Under most climatic and edaphic conditions, atrazine (2-4 lb/acre) has residual phytotoxicity for 4-7 months (Harris and Sheets 1965). Residual carryover after repeated application is minimal.

Analysis of surface, subsurface, and finished waters in Iowa, where atrazine is widely used in corn production, indicated that the herbicide was present in small amounts (<50 µg/L). Atrazine levels in surface waters correlated with discharge volume data for the river (Richard et al. 1975).

Laboratory and field tests have indicated that atrazine is moderately toxic to fish in comparison to other herbicides. Macek et al. (1976b) investigated the effects of atrazine on survival, growth, and reproduction of three species of fish. Utilizing soft water (hardness 33 to 40 mg/L) and a continuous flow apparatus, Macek et al. were able to show in acute toxicity tests that both the 96-h and incipient LC50 for fathead minnows were 15 mg/L atrazine (95% CI 11-20). Their acute 96-h LC50 for bluegills was >8.0 mg/L and the incipient LC50 was 6.7 (5.4-8.4) mg/L atrazine, which agrees with the 96-h LC50 of approximately 6 mg/L atrazine (wetable powder) reported by Walker (1964) for this species. The 96-h LC50 for atrazine toxicity to brook trout reported by Macek et al. (1976b) was 6.3 mg/L (4.1-9.7) and the incipient LC50 was 4.9 mg/L (4.0-6.0). This is similar to the 48-h LC50 (12.6 mg/L) reported for rainbow trout in a static bioassay (FWPCA 1968).

Bluegill and fathead minnow spawning, survival, and growth were not affected by exposure to 0.095 and 0.213 mg/L atrazine, respectively (Macek et al. 1976b). Hiltibran (1967) found that 10 mg/L granular atrazine did not affect green sunfish embryo development, or bluegill and green sunfish survival over 8 days. Lake chub sucker fry *(Ermyzon sucretta)* survived 10 mg/L wetable powder atrazine. Similarly, brook trout parental survival, egg production, and hatchability appeared to be unaffected by exposure to $\leq$0.72 mg/L atrazine (Macek et al. 1976b). Survival and growth of brook trout fry were, however, significantly reduced following 90 days of exposure to 0.72, 0.45 and 0.24 mg/L atrazine. Analysis of muscle tissue from bluegills, fathead minnows, and brook trout indicated that these fish bioconcentrated detectable amounts of atrazine after prolonged exposure (Macek et al. 1976b).

Walker (1964) observed no fish mortality after application of 2.0 to 6.0 mg/L atrazine to ponds infested by aquatic weeds. He suggested, however, that atrazine had the potential to affect fish in ways other than direct toxicity. A reduction in bottom fauna was observed immediately following application. Among the most sensitive species were mayflies *(Ephemeroptera)*, caddisflies *(Tricoptera)*, leeches *(Hirudinea)* and gastropods *(Musculium)*. Studies by Macek et al. (1976b) on the chronic toxicity of atrazine to selected aquatic invertebrates indicated that morphological development of progeny is particularly sensitive. Exposure of two successive generations of chironomids to 0.23 mg/L atrazine resulted in reduced hatching success, larval mortality, developmental retardation, and a reduction in the percentage of pupating larvae and emerging adults. Continuous exposure to 0.25 mg/L atrazine significantly reduced production of *Daphnia*. Development to the seventh instar of F_1 gammarids exposed to 0.14 mg/L atrazine was reduced 25% below that of lower concentrations and controls.

Herbicidal destruction of aquatic vegetation may expose small forage fish to predation by large predacious fishes. Furthermore, fluctations in oxygen tensions have frequently been shown to occur after application of triazine herbicides. These fluctuations were associated with phytoplankton blooms occurring in conjunction with decomposition of submerged vegetation (Walker 1964).

Experimental Results

In our toxicity test, atrazine (AAtrex) appeared to produce a gradual concentration dependent mortality in freshwater, with losses of 5 and 25% at concentrations of 8 and 15 mg/L, respectively (Table 6). Dead fish showed signs of severe edema. No apparent affect on (Na,K)-stimulated ATPase activity occurred. McBride and Richards (1975) found that atrazine significantly decreased sodium uptake of isolated perfused gills from carp *(Cyprinus carpio)* but did not affect fluid flow rate at the concentrations tested. The authors indicated the effect of atrazine on (Na,K)-ATPase systems should be studied, because other pesticides (aldrin and DDT) have been shown to inhibit (Na+K)-ATPase (Cutkomp et al. 1971, Koch et al. 1971). When the survivors were transferred to seawater, the group that had been exposed to 15 mg/L atrazine suffered a 25% mortality (Table 6). The deaths occurred within the first 24 h and probably resulted from the poor condition of the fish (due to toxicant exposure) and not osmoregulatory failure. Histological examination of three fish exposed to 15 mg/L atrazine showed no apparent affect on liver or kidney tissues but hypertrophy of gill epithelium was evident in two of the fish (Appendix II).

TABLE 6. SURVIVAL AND GILL ATPASE OF YEARLING COHO SALMON EXPOSED TO ATRAZINE IN FRESHWATER AND THE SUBSEQUENT SURVIVAL FOLLOWING TRANSFER TO SEAWATER (MAR. 2-19, 1977).

Concentration (mg/L) nominal	measured	Percent survival[a] (144-h exposure FW)	Gill ATPase[b]	Percent survival (264-h sw)
Control	0	100	2.8	100
0.25	0.21-0.23	100	NT[c]	100
0.50	0.48-0.51	100	NT	100
1.0	1.31-1.38	100	3.4	100
3.0	3.85-4.25	100	NT	100
5.0	4.47-5.62	100	NT	100
8.0	10.65	95	2.4	94
15.0	18.0-18.8	75	2.3[d]	75

[a] *Twenty fish exposed per concentration.*
[b] *(Na,K)-stimulated ATPase activity of gills; mean of 5 fish.*
[c] *Not measured.*
[d] *Mean of 3 fish.*

DICAMBA

Review of Literature: Toxicity to Fish and Behavior in the Environment

Dicamba (3,6-dichloro-o-anisic acid) is one of the most extensively used benzoic acid herbicides in the United States. Production of dicamba in 1971 was estimated to be 6 million lbs of active ingredient (Lawless et al. 1972). The herbicide was introduced in the early 1960's for the control of preemergence and postemergence broadleaf weeds in cereal grains (Frear 1976 and Velsicol Chem. Co. 1967). Phenoxy-tolerant broadleaf weeds and brush species are also controlled by foliar and area applications of dicamba. Current registration for this herbicide is for use on barley, corn, oats, wheat, pasture and rangeland, where it is applied at rates ranging from 1/4 to 8 lb/acre. The dimethylamine salt of dicamba, formulated as a liquid, is sold under the trade name of Banvel, but is also available in granular form as the acid or amine salt.

Dicamba has been shown to exhibit intermediate persistence in many soil types when compared to other herbicides (Burnside et al. 1971), remaining phytotoxic for several months (Klingman and Ashton 1975). It has a relatively high water solubility (7900 mg/L) and has been demonstrated to move within the soil profile with water flux. Studies by Trichell et al. (1968) have shown that runoff losses of dicamba are limited. The vapor pressure of dicamba is quite low, so minimal amounts are lost through volatility (Montgomery et al. 1976). Photodecomposition of dicamba is similarly limited. Indirect evidence from a number of studies suggests that microbial degradation may be instrumental in reducing dicamba persistence (Frear 1976 and Hahan et al. 1969). Norris and Montgomery (1975) noted that following spraying of a brushy area in coastal Oregon with 1.12 kg dicamba/ha, water residue levels rose sharply to 37 ppb about 5 h after spraying and then declined slowly to background levels by 37.5 h.

The effects of dicamba on fish have not been well investigated. Toxicity tests with dicamba indicate a low toxicity to salmonids and warm water fish species. In static tests conducted by Bond et al. (1965) the 24 and 48-h LC50 values for juvenile coho salmon were 151 and 120 mg/L active ingredient, respectively (methyl orange alkalinity approximately 55 mg/L, pH about 7.7). Rainbow trout were killed by a concentration of 320 mg/L dicamba in 72 h (Bond et al. 1965). Bohmont (1967), in his literature review of Cope's 1962 and 1963 work, however, reported an estimated 48-h LC50 for rainbow trout and bluegill of 35.0 mg/L and 130 mg/L dicamba (Banvel D), respectively. Hughes and Davis (1962b) reported 24 and 48-h LC50 values of 600 and 410 mg/L for the liquid formulation of dicamba for the bluegill; however, when Banvel D acid was adsorbed onto vermiculite they found a 24-h LC50 of 20 mg/L.

Experimental Results

No mortalities were observed in yearling coho salmon exposed to dicamba (Banvel) concentrations up to 100 mg/L (Table 7). Gill (Na,K)-stimulated

ATPase activity appeared unaffected by the herbicide. Histological examination of gill, liver, and kidney tissue indicated no apparent effect of exposure to dicamba (Appendix II). Following 144-h exposure to the toxicant, the yearling coho salmon were challenged with seawater. Fish previously exposed to the lowest concentration, 0.25 mg/L dicamba, showed a 32% mortality during the 11 days of the seawater challenge (Table 7), however, no deaths occurred at higher concentrations. The deaths began after 96 h in seawater and concluded at about 200 h. No explanation is available for this unusual pattern of mortality. The deaths followed the time pattern of osmoregulatory failure noted in previous seawater challenge tests following copper exposure (Lorz and McPherson 1977).

TABLE 7. SURVIVAL AND GILL ATPASE OF YEARLING COHO SALMON EXPOSED TO DICAMBA IN FRESHWATER AND SUBSEQUENT SURVIVAL FOLLOWING TRANSFER TO SEAWATER (JAN. 6-23, 1977).

Concentration (mg/L) nominal	measured	Percent survival[a] (144-h exposure FW)	Gill ATPase[b]	Percent survival (268-h SW)
Control	0	100	2.52	100.0
0.25	0.19- 0.22	100	NT[c]	68.4
0.50	0.40- 0.42	100	NT	100.0
1.0	0.54- 0.56	100	NT	100.0
5.0	3.15- 3.33	100	1.64	100.0
10.0	10.05-10.11	100	NT	100.0
50.0	50.5 -53.2	100	2.04	100.0
100.0	108.2-109.9	100	2.20	100.0

[a] *Twenty fish exposed per concentration.*
[b] *(Na,K)-stimulated ATPase activity of gills; mean of 5 fish.*
[c] *Not measured.*

KRENITE

Review of Literature: Toxicity to Fish and Behavior in the Environment

Krenite is a postemergence herbicide containing 41.5% (4 lb a.i./gal) ammonium ethyl carbamoylphosphonate. It is registered for brush control on non-cropland areas such as railway and highway right-of-ways. drainage ditch banks, industrial plant sites, and other similar areas, including use around domestic water supply reservoirs, lakes, and ponds. Krenite brush control agent is absorbed and translocated by plants after foliar application, but little effect is evident until the following year when susceptible plants fail to leaf out. For this reason application is recommended 2 months prior to fall leaf coloration at the rates of 3-5 lb/acre or 4 lbs/100 gal water for spot treatment.

Krenite has a high water solubility, is nonvolatile, and is rapidly inactivated in the soil (Oregon Weed Control Handbook 1977). Soil residue

studies have indicated that, in 2 weeks, half of the herbicide is converted to carbamoyl phosphonic acid which is subsequently converted to CO_2 and humic acid fractions within 8 weeks. Bottom sediments lose Krenite in 3 months or less (Dr. James Harrod, duPont Co., unpublished report).

There is little published data on the toxicity of Krenite to fish. Unpublished findings of E. I. duPont de Nemours and Company, Inc. (technical pamphlet) indicate that rainbow trout and fathead minnow have a 96-h LC50 of 1,000 mg/L (product), while bluegill sunfish exhibit a 96-h LC50 of 670 mg/L (product). Laboratory tests have demonstrated that Krenite is not bioaccumulated. Residues in fish tissues were comparable to the concentration of the herbicide in the water (Newton and Norgren 1977).

Experimental Results

No mortalities were observed in yearling coho salmon exposed to Krenite concentrations up to 200 mg/L. When survivors were transferred to seawater only minimal mortality occurred (Table 8). Krenite is not very toxic to coho salmon, as neither freshwater survival nor subsequent seawater survival were affected. There was no apparent effect of Krenite on the (Na,K)-stimulated ATPase activity of the gill (Table 8).

TABLE 8. SURVIVAL AND GILL ATPASE OF YEARLING COHO SALMON EXPOSED TO KRENITE IN FRESHWATER AND THE SUBSEQUENT SURVIVAL FOLLOWING TRANSFER TO SEAWATER (FEB. 2-17, 1977).

Concentration (mg/L) nominal	Percent survival[a] (144-h exposure FW)	Gill ATPase[b]	Percent survival (216-h SW)
Control	100	2.2	100
0.25	100	1.8	93
0.50	100	NT[c]	100
1.0	100	1.7	100
10.0	100	NT	100
50.0	100	NT	100
100.0	100	NT	100
200.0	100	2.7	100

[a] *Twenty fish exposed per concentration.*
[b] *(Na,K)-stimulated ATPase activity of gills; mean of 5 fish.*
[c] *Not measured.*

PARAQUAT

Review of Literature: Toxicity to Fish and Behavior in the Environment

Paraquat is the common name for 1,1'-dimethyl-4,4'-bipyridinium ion. It is formulated under the trade names Ortho^R Paraquat and Gramozone^R.

Paraquat formulations are presently available as chloride salts (Lawrence et al. 1965). The pure chloride salt is a white solid that forms a dark red aqueous solution. Paraquat is a nonselective, quick-acting herbicide and desiccant, and is used extensively due to its effectiveness on grasses and most broadleaf weed species (Calderbank and Slade 1976). Paraquat is registered for use as a directed spray on preplant treatment in many tree crops and several field crops including alfalfa, corn, soybeans, sugar beets, and tomatoes. It is also employed as a preharvest desiccant on cotton, potatoes, and soybeans, and in aquatic weed control. Normal application rates for paraquat are 1/4 to 1 lb (a.i.)/acre, and 0.1 to 1.5 mg/L in the aquatic environment (Calderbank and Slade 1976).

Paraquat dichloride is a quarternary ammonium salt, highly soluble in water, insoluble in most organic solvents, and nonvolatile. The herbicide has little or no residual activity due to its rapid inactivation by irreversible adsorption to soil. The persistence of paraquat in water is highly variable and dependent upon water movement, weed density, and the presence of mud, suspended silt, and sunlight. Herbicide residues are normally reduced to 0.01 mg/L or less in 6 to 14 days. Herbicide adsorbed to the hydrosol persists for long periods but is probably not biologically active (Calderbank and Slade 1976). Grzenda et al. (1966) found that paraquat applied to ponds at rates of 2.1 and 2.5 mg/L persisted in the water for 6 and 23 days, respectively. No desorption of paraquat from the bottom clays was observed 3 months after initial application.

Burnet (1972) found that amphipods were reduced to 5% of pretreatment levels in drift fauna following treatment of a New Zealand stream with 2 mg/L paraquat.

Paraquat and diquat do not appear to be bioconcentrated in fish. Calderbank and Slade (1976) reported on some unpublished findings of Carter (1971). Rainbow trout exposed to these herbicides (1.0 mg/L) for 16 days accumulated 0.5 to 0.6 mg/L whole body residues of either herbicide. Residues were detected in the skin, gills, gut, and organs, but not in the muscle. Upon transfer of the fish to freshwater the residues steadily declined. Similar findings for whole-body residues of diquat and paraquat were reported by Cope (1966). When several ponds were treated with 1 mg/L paraquat (emulsion) or diquat, paraquat bioaccumulation was detected in bluegill sunfish (1.21 mg/L whole-body residues) but little diquat bioaccumulation was detected (0.09 mg/L). Residues of paraquat in rainbow trout, green sunfish, and channel catfish *(Ictalurus punctatus)* were 0.37 mg/L or less.

Earnest (1971) found that paraquat levels ranged from 0.58 mg/kg in green sunfish to 1.86 mg/kg in rainbow trout 1 day after treatment of a Colorado pond. Residue levels in bluegills reached a maximum of 1.58 mg/kg 8 days after treatment and then declined.

A comparison of normal paraquat usage levels with fish toxicity suggests that the direct poisoning of fish by field application is a remote possibility. Bluegills, largemouth bass, fathead minnows, channel catfish, and rainbow trout exhibit approximate threshold (LC10) toxicity levels of 5 mg/L

paraquat (cation) for a 96-h contact period (Lawrence et al. 1965). Davis and Hughes (1963) reported a 48-h LC50 for bluegill sunfish as 100 mg/L paraquat (cation). Under static conditions (pH 7.6 to 8.0, hardness 210 to 290 mg/L), brown trout had a 48-h LC50 with paraquat of 82 mg/L (Woodiwiss and Fretwell 1974). Alabaster (1969) reported a 48-h LC50 for harlequin fish as 32 mg/L paraquat using a flow-through technique and water of 20 mg/L hardness. Butler (1965) exposed the estuarine longnose killifish *(Fundulus similes)* to 1.0 mg/L paraquat and found no effect.

Information on paraquat toxicity to fish under field conditions is limited. Yeo (1967) reported that smallmouth bass and mosquito fish were killed when placed in 180-gallon plastic pools with 1.0 and 3.0 mg/L paraquat (pH 9.4). Blackburn and Weldon (1962), however, reported that paraquat applied to a small Florida canal at 1.0 mg/L was not toxic to fish (water temperatures 29 to 33°C, pH 7.6). Earnest (1971) noted that a minimum of 34% of bluegills, placed in a Colorado farm pond, died within 48 h after treatment with 1.14 mg/L paraquat (surface temperature 18.9-25.0°C, alkalinity 69-129 mg/L and pH 8.0-10.4).

Newman and Way (1966) reported no direct effects of paraquat or diquat on aquatic invertebrates in their experiments. They did, however, note severe oxygen depletion at one location following decay of the treated aquatic weeds. A low oxygen content is postulated to have caused the deaths noted among Hirudinea, Isopoda, Odonata, Coleoptera, Trichoptera, Gastropoda, Lamellibranchiata and captive trout, although free-living coarse fish and trout appeared unaffected.

Experimental Results

In our study the 96-h LC50 of paraquat-Cl was 76 mg/L for yearling coho salmon. Deaths occurred in a dose-dependent manner depending on concentration and exposure time (Fig. 5). When surviving fish were transferred to seawater, all coho salmon previously exposed to 50 mg/L died during the first 40 h of exposure. Similarly, 64% of the fish previously exposed to 10 mg/L died during the first 68 h in seawater (Table 9). Our 96-h LC50 value of 76 mg/L is slightly lower than the 48-h LC50 of 82 mg/L given for brown trout by Woodiwiss and Fretwell (1974). The different species and water quality, however, of the two test solutions could account for this. The (Na,K)-stimulated ATPase activity of the gills was not affected by exposure to paraquat-Cl. Histological examination of fish exposed to 100 mg/L paraquat-Cl for 120 h showed evidence of degenerative damage to gills, and kidneys, and slight necrotic areas in the liver (Appendix II). Earnest (1971) similarly reported necrosis of liver tissue following treatment of the Colorado farm pond with paraquat.

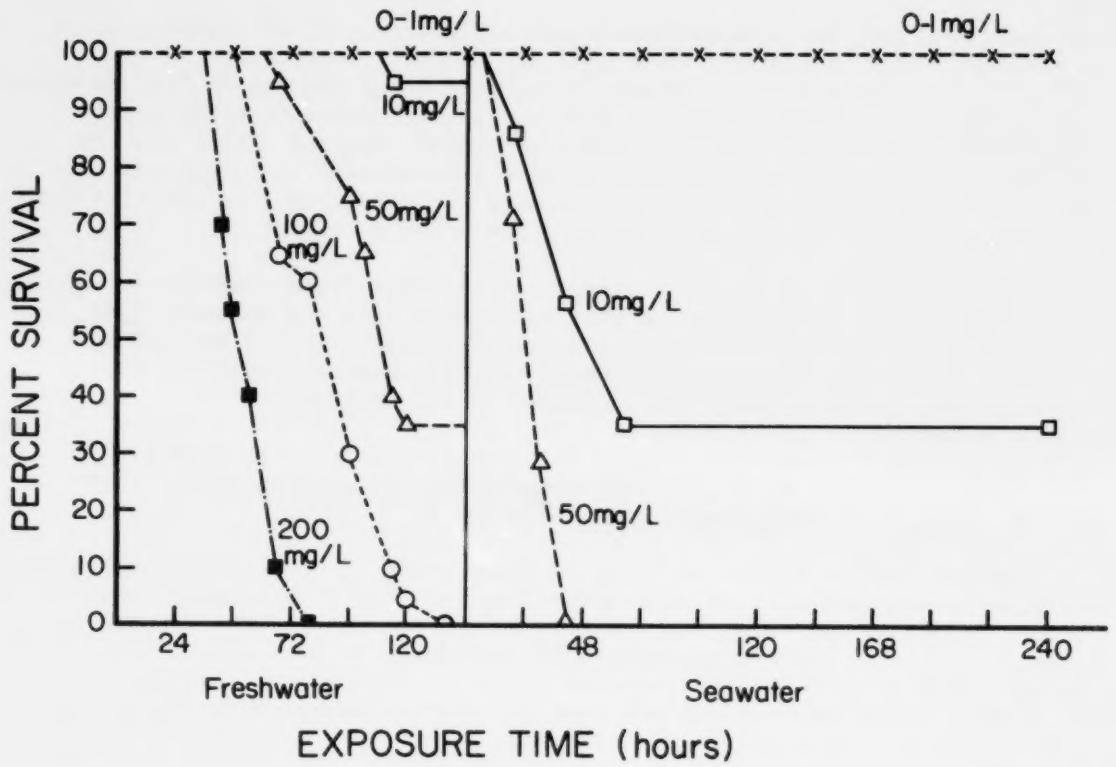

Figure 5. Percent survival of yearling coho salmon during exposure to paraquat-CL in freshwater and subsequent survival upon transfer to seawater.

TABLE 9. SURVIVAL AND GILL ATPASE OF YEARLING COHO SALMON EXPOSED TO PARAQUAT-CL IN FRESHWATER AND THE SUBSEQUENT SURVIVAL FOLLOWING TRANSFER TO SEAWATER (MAR. 22-APR. 7, 1977).

Concentration (mg/L) nominal	measured	Percent survival[a] (144-h exposure FW)	Gill ATPase[b]	Percent survival (240-h SW)
Control	0	100	3.04	100
0.25	0.14- 0.19	100	NT[c]	100
0.50	0.50- 0.52	100	4.46	100
1.0	1.08- 1.12	100	1.96	100
10.0	10.6	95	1.90	36
50.0	56.3	35	NT	0
100.0	112.7-113.7	0		NT
200.0	238.7-251.2	0		NT

[a] *Twenty fish exposed per concentration.*
[b] *(Na,K)-stimulated ATPase activity of the gills; mean of 5 fish.*
[c] *Not measured.*

2,4-D

Review of Literature: Toxicity to Fish and Behavior in the Environment

The chlorine-substituted phenoyxacetic acids, 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) were introduced as selective herbicides following investigation of their growth-regulating and herbicidal properties during World War II (Templeman 1955). They are effective against many broadleaf weeds but not against graminaceous weeds. The herbicide 2,4-D is registered for use on forests, range and pasture grasses, rye, wheat, barley, oats, corn, asparagus, apples, pears, grapes, potatoes, blueberries, cranberries, non-cropland uses, turf, and certain aquatic sites.

Most phenoxyalkanoic acid herbicides are formulated as the salt or ester form. Amines of 2,4-D are the most commonly used salt forms of 2,4-D, although the sodium, potassium and ammonium salts are also used. The di-and tri-substituted amines are particularly important; they are highly soluble in water and are used in the formulation of water soluble concentrates. Normal rates of application of 2,4-D amines are as follows: 1/4 to 1-1/2 lb/acre (a.i.) for most crops; 2 to 3 lb/acre (a.i.) for forest spraying; and 0.25 to 0.50% (a.i.) for the control of emergent and floating weeds. The ester formulations of 2,4-D are extremely important and are used extensively in forestry and agriculture for vegetation control. The ester formulations are 10 to 100 times more toxic to fish and other aquatic organisms than the dimethylamine salt, but are often chosen for use because of desirable physical properties such as: control of droplet size, limited solublity in water, good spread of herbicide upon contact with vegetation being controlled, and persistence in the environment.

The salts of 2,4-D have very low volatility. When dissolved in hard water they form insoluble and essentially inactive calcium, magnesium, or iron salts of 2,4-D. Leaching of 2,4-D in soil is dependent upon soil type and herbicide solubility in water. Decreased leaching occurs in clay and organic soils due to adsorption by soil colloids.

Crosby and Tutass (1966) found that 2,4-D decomposed rapidly in the presence of water and ultraviolet light or sunlight, and they identified the decomposition products. Audus (1950) demonstrated the importance of microbial degradation of phenoxyacetic acid herbicides. In a warm, moist loam, 2,4-D can be expected to disappear within 2 to 3 weeks (Loos 1976). Norris (1970) noted that 94% of 2,4-D was degraded in 35 days when applied in a red alder forest floor.

Wojtalik et al. (1971) found no harmful or distinguishable response or accumulation in zooplankton, phytoplankton or macroinvertebrates following treatment of water with 20 to 40 lb per acre (acid equivalent, a.e.) of DMA-2,4-D. The authors believed there was little danger of biomagnification of the 2,4-D in contrast to chlorinated hydrocarbon pesticides. Wojtalik et al. reported residues (in water) greater than 0.02 mg/L at only 2 of 19 stations 4 weeks after treatment. The authors stated that disappearance of DMA-2,4-D from water is rapid compared with the herbicides dichlobenil and fenac which persisted at detectable levels for up to 160 days.

Residues of the dimethylamine salt of 2,4-D (DMA-2,4-D) in hydrosol and fish following pond application have been reported by Schultz and Harman (1974a). Nine ponds in Florida, Georgia, and Missouri were treated with either 2.24, 4.48 or 8.96 kg/ha of the herbicide. The highest detectable level of 2,4-D in water (0.692 mg/L) was found 3 days after application in a pond in Georgia that had been treated with 8.96 kg/ha DMA-2,4-D. The herbicide had decreased to trace amounts (less than 0.005 mg/L) 28 days after application and was not detected thereafter. The highest detectable level of DMA-2,4-D in mud occurred in the Georgia pond, seven days after treatment. Largemouth bass, channel catfish, bluegill, and redear sunfish, *Lepomis microlophus*, were held in live cages during and after pond treatment; no mortality was observed in any of the ponds. Fifteen percent of the fish sampled contained detectable residues of DMA-2,4-D (1.0 to 0.010 mg/kg). Radiometric measurements of the uptake, metabolism, and dissipation of the DMA salts of ring-labeled C^{14}-2,4-D in water, hydrosol, and fish are in general agreement with these findings (Schultz 1973). Catchable-size catfish, bluegills, and largemouth bass were exposed to 0.5 to 2.0 mg/L DMA-2,4-D for 84 days in plastic pools. Muscle tissue of fish exposed to an initial treatment of 2 mg/L DMA-2,4-D contained less than 0.06 mg/L 2,4-D, at the completion of the experiment (84 days). Thirty-five days after treatment the highest detectable level in the water was 0.05 mg/L 2,4-D. The greatest residue in the hydrosol, 0.21 mg/L, occurred after 7 days in a pond treated with 2.0 mg/L DMA-2,4-D. Accumulations of 2,4-D in fish tissues in order of the highest to lowest concentration were: channel catfish, bile>liver>kidney>gill>brain >muscle; and bluegill sunfish, bile>pyloric caeca>kidney>liver>gill>brain >muscle (Schultz 1971).

Norris and Moore (1971) reviewed the fate of chemicals in the aquatic environment and indicated that chemicals may be lost through volatilization, adsorption on stream sediments, absorption by aquatic biota, degradation by chemical, biological, or photochemical means, or by downstream movement. They noted that the literature contains conflicting reports of the persistence of pesticides in ponds and streams. Aly and Faust (1964) reported that 2,4-D esters were hydrolyzed to the free acid in 9 days in lake water, but 2,4-D acid persisted up to 120 days. Schwartz (1967), however, noted only 40% degradation of 2,4-D in water in 6 months under excellent conditions for biological activity. Norris (1967) noted that herbicide residues were found for only short periods of time in streams within or adjacent to forest treatment areas. Concentrations approaching 1 mg/L have been found in puddles which collected the first significant runoff from an area treated one month earlier (Norris 1978). However, in 6 years of monitoring spray operations for residues, phenoxy herbicides never exceeded 0.1 mg/L in western Oregon streams (Norris and Moore 1971).

A review of the toxicity of DMA-2,4-D herbicides indicates that they are relatively low in toxicity to fish. Folmar (1976) reported a 96-h LC50 for rainbow trout at 100 mg/L. Davis and Hughes (1963) and Hughes and Davis (1963) tested the toxicity of different formulations of 2,4-D to bluegills. They found considerable variation in the toxicity of different formulations and even in the toxicity of a single formulation. The researchers felt that these inconsistencies could be attributed to the different batch lots and/or manufacturing plants. The alkanolamine salt

and the dimethylamine formulations were the least toxic to bluegills of 11 formulations of 2,4-D tested (800-166 mg/L as 48-h LC50 depending on batch). The isopropyl ester and butyl ester were the most toxic (0.8 and 1.3 mg/L as 48-h LC50 concentrations, respectively, for bluegills). Davis and Hardcastle (1959) found differences in LC50 values for 2,4-D and other herbicides when waters from two different sources were used in toxicity tests.

Schultz and Harman (1974b) reviewed the literature concerning aquatic use of 2,4-D compounds as a prerequisite for registration of 2,4-D for use on irrigation canal banks and for use in moving water. They noted that many formulations of 2,4-D are available, but the one most commonly used in aquatic situations is the dimethylamine salt of 2,4-D (DMA-2,4-D). The ester formulations have also been used, but are many times more toxic to fish and other aquatic organisms than the dimethylamine salt. Meehan et al. (1974) tested the toxicity of various formulations of 2,4-D to salmonids and noted that <50 mg/L 2,4-D acid produced no mortality except in pink salmon fry. The butyl ester, however, was very toxic causing almost complete mortality in all species at concentrations >1 mg/L. The isooctyl ester was the least toxic of the ester formulations. Meehan concluded that specific phenoxy herbicide ester formulations should be chosen with regard to their impact on aquatic organisms if there is a possibility that the chemical will enter the water.

Rodgers and Stalling (1972) exposed rainbow trout, channel catfish and bluegills to a C^{14} labeled butoxyethanol ester of 2,4-D and studied its uptake from the water by fed and fasted fish. Maximum residue concentrations were observed in most organs of fed fish within 1-2 h of exposure and within 1-8 h exposure for fasted fish. The herbicide or its metabolites were eliminated rapidly after maximum residue concentrations were reached. In a large scale application of 2,4-D (butoxyethanol ester) in seven TVA reservoirs, Smith and Isom (1967) found little uptake of 2,4-D by fish but some by mussels during 96 h of exposure. Significant concentrations of 2,4-D, however, were noted in isolated sediment samples up to 10 months after treatment.

Histological and biochemical changes were observed in bluegill sunfish exposed to 2,4-D, Esteron 99 (propylene glycol butyl ether ester), in ponds in Oklahoma (Cope et al. 1970). The pathology involved liver, vascular system, and brain, with depletion of liver glycogen, globular deposits in the blood vessels and stasis and engorgement of the brain circulatory system.

Experimental Results

In our study, no mortalities were observed when yearling coho salmon were challenged with up to 200 mg/L of 2,4-D (DMA) for 144 h (Table 10). Similarly, no affect on the (Na,K)-stimulated ATPase activity of the gills was noted. When coho salmon previously exposed to 2,4-D were challenged with seawater little mortality occurred (Table 10). Therefore, comparison of our test results to those of Meehan et al. (1974) indicates that it would be safest to use the DMA formulation of 2,4-D if the desired herbicidal effects could be achieved.

TABLE 10. SURVIVAL AND GILL ATPASE ACTIVITY OF YEARLING COHO SALMON EXPOSED TO 2,4-D IN FRESHWATER AND THE SUBSEQUENT SURVIVAL FOLLOWING TRANSFER TO SEAWATER (DEC. 29, 1976-JAN. 14, 1977).

Concentration (mg/L) nominal	measured	Percent survival[a] (144-h exposure FW)	Gill ATPase[b]	Percent survival (240-h SW)
Control	0	100	1.4	100.0
0.25	0.25- 0.18	100	NT[c]	94.8
0.50	0.52- 0.59	100	NT	100.0
1.0	1.07	100	1.6	100.0
10.0	10.4	100	NT	100.0
50.0	59.6- 64.0	100	NT	100.0
100.0	106.8-115.4	100	1.0	100.0
200.0	170.0-237.0	100	1.3	100.0

[a] *Twenty fish exposed per concentration.*
[b] *(Na,K)-stimulated ATPase activity of the gills; mean of 5 fish.*
[c] *Not measured.*

2,4,5-T

Review of Literature: Toxicity to Fish and Behavior in the Environment

The herbicide 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) is identical to 2,4-D with the exception of an additional chlorine atom on the number 5 carbon in the ring. Many of the roses, legumes, and broadleaved plants that are resistent to 2,4-D are controlled with 2,4,5-T. The herbicide has been cleared for use on pastures, rangelands, right-of-way, and forests. It is not registered for use on food crops nor for use in water, on ditch banks, around homes or recreational areas, or near populated areas. The herbicide 2,4,5-T is normally applied at 1 to 2 lb/acre (a.i.) for area treatment or at 2 to 4 lb/gal (a.i.) per 100 gallons of solution for spot treatment.

The water solubility of 2,4,5-T (238 mg/L) is less than that of 2,4-D [(600 mg/L) (Montgomery et al. 1976)], and this influences its pattern of use. The sodium salt of 2,4,5-T, for example, unlike that of 2,4-D, has a low solubility and is difficult to get into a herbicidally active solution. Amine salts have been shown to form complexes with sodium, iron, calcium, and magnesium.

Under conditions favorable for microbial degradation, 2,4,5-T has a longer residual life in soil than 2,4-D. Normal persistence of 2,4,5-T in soils is from 45 to 270 days (Newman et al. 1952). Norris et al. (1977) discussed the persistence of 2,4,5-T in a Pacific Northwest forest. The 2,4,5-T level (in the forest floor) declined 90% during the first 6 months following application, and there was less than 0.02 kg/ha remaining after 1 year or 0.76% of the originally applied 2,4,5-T. Other properties of the ester and amine formulations are comparable to those of 2,4-D.

Data on toxicity of triethylamine salts of 2,4,5-T to aquatic organisms are scarce. Kenaga (1974) reviewed the literature concerning the toxicity to fish of 2,4,5-T and its derivatives. Exposure to the commercial formulation DED-WEED^{R} at concentrations >72 mg/L (a.e.) for 24 and 96-h resulted in at least 50% mortality of bluegill sunfish, channel catfish, and fathead minnows. Exposure of rainbow trout to the same formulation [>72 mg/L DED-WEED^{R} (a.e.)] resulted in 50% mortality in 24 h whereas at concentrations of 0.07-0.72 mg/L (a.e.) for 96 h exposure Kenaga also reported mortality rates of 50%. Spot *(Leiostomus xanthurus)* exposed to 0.4 mg/L (a.e.) for 24 h showed no mortalities. The fathead minnow exposed to 40.2 mg/L for 72 h had no mortality, but at concentrations >72 mg/L for 24 or 96 h at least 50% mortality was observed (Kenaga 1974). Davis and Hughes (1963), utilizing the triethylamine salt of 2,4,5-T (Crop Rider^{R}) found the 24 and 48-h LC50 for bluegill sunfish to be approximately 53 mg/L (a.e.).

Experimental Results

No deaths were observed in yearling coho salmon exposed to concentrations of 6-7 mg/L of 2,4,5-T (triethylamine salt, Weedar) for 144 h (Table 11). Similarly, there was no apparent effect on (Na,K)-stimulated ATPase activity of the gills. When the fish were placed in seawater only minimal mortality occurred (Table 11). The lack of mortality during the toxicant exposure was surprising considering that concentrations of 0.07-0.72 mg/L (a.e.) were reported to cause 50% mortality in 96 h to rainbow trout (Kenaga 1974); however, no water quality data were presented. Generally, LC50 values for herbicides obtained at 24 h have been about 10 times greater than the LC50 concentration noted for 96-h exposure.

TABLE 11. SURVIVAL AND GILL ATPASE OF YEARLING COHO SALMON EXPOSED TO 2,4,5-T (WEEDAR) IN FRESHWATER AND THE SUBSEQUENT SURVIVAL FOLLOWING TRANSFER TO SEAWATER (MAR. 31-APR. 17, 1977).

Concentration (mg/L)		Percent survival[a]	Gill ATPase[b]	Percent survival
nominal	measured	(144-h exposure FW)		(240-h SW)
Control	0	100	4.06	100
0.05	0.016-0.021	100	NT[c]	95
0.10	0.041-0.043	100	NT	100
0.25	0.13 -0.15	100	NT	100
0.50	0.27	100	NT	100
1.0	0.57 -0.74	100	3.52	100
10.0	6.64 -7.0	100	4.66	100

[a] *Twenty fish exposed per concentration.*
[b] *(Na,K)-stimulated ATPase activity of the gills; mean of 5 fish.*
[c] *Not measured.*

ESTERON BRUSH KILLER

Review of Literature: Toxicity to Fish and Behavior in the Environment

Esteron[R] Brush Killer contains equal concentrations of 2,4-D and 2,4,5-T [2 lb each/gal (a.e.)] as the propyleneglycol butyl ether (PGBE) ester. This ester of 2,4-D and 2,4,5-T is very soluble in oil and organic solvents but has very low solubility in water. However, in the presence of emulsifiers and with agitation, an emulsion is formed in water; Esteron Brush Killer is applied in this manner.

The 2,4-D esters of low-molecular-weight alcohols have an appreciable vapor pressure (Loos 1976). The long-chain alcohols with an ester linkage are less volatile and present less of a hazard to non-target plants and animals.

The distribution and absorption of these ester formulations is also influenced by their rate of degradation to the parent compound. The speed of hydrolysis of 2,4,5-T esters has been related to pH. Alkaline conditions are more conducive to deesterification of these compounds than are acidic conditions (Kenaga 1974). Teasley and Williams (1969, in Kenaga 1974) studied degradation rates of a number of 2,4,5-T esters including the PGBE ester formulation. In water of pH 6.5 all of the herbicides exhibited similar rates of deesterification at a concentration of 1 mg/L (a.e.). Hydrolysis rates for 2,4-D esters should be faster than those observed for 2,4,5-T esters of comparable structure.

Although most salts of 2,4-D and 2,4,5-T are low in toxicity to fish, certain ester formulations of these herbicides are toxic to some species at 1 mg/L (Meehan et al. 1974, Kenaga 1974). Particularly toxic to fish are PGBE esters, butoxyethanol (BE) esters, and butyl esters. The 48-h LC50 of rainbow trout to the PGBE ester of 2,4-D and 2,4,5-T was 980 and 570 µg/L, respectively (FWPCA 1968).

Much of the increased toxicity of ester formulations over that of their parent compounds can be explained by differences in their physical and chemical properties. The 2,4,5-T esters have high partition coefficients, which are related to the greater absorption of the compounds into fatty tissues such as skin and gills. The PGBE, BE, butyl, and isopropyl esters of 2,4,5-T have relatively high partition coefficients, which may partially explain their high toxicity to fish (Kenaga 1974).

Sublethal effects of PGBE esters of 2,4-D have been demonstrated for fish (Cope 1966). Spawning of bluegill sunfish was delayed 2 weeks in ponds treated with 5 and 10 mg/L of the herbicide. Hiltibran (1967) observed that fertilized eggs of green sunfish developed normally when exposed to 1 mg/L of the PGBE ester of 2,4-D under static water conditions. Bluegill, green sunfish, lake chub suckers, and smallmouth bass fry, however, appeared to be more susceptible to the herbicide, as they failed to survive the 8-day duration of the test.

Much of the fish toxicity work on the phenoxy herbicides concerns the PGBE esters of 2,4-D or 2,4,5-T, but little has been done on mixtures of these compounds. Hughes and Davis (1962b) found that both the 24 and 48-h LC50's for bluegill sunfish exposed to the PGBE ester of 2,4-D were 29 mg/L under static conditions in water with a mean pH of 6.9 and a mean hardness of 29 mg/L. Meehan et al. (1974) observed that the 96-h no-effect level for coho salmon fingerlings exposed to the PGBE ester of 2,4-D was less than 1 mg/L. There was a mean fry mortality of 26.7% after 96 h of exposure to 1 mg/L of the herbicide in water that ranged in hardness from 10.0 to 33.6 mg/L as calcium plus magnesium. A 48-h LC50 of 1.1 mg/L PGBE ester of 2,4-D was reported by Cope (1966) for rainbow trout (no water quality given). Butler (1965) observed that the 48-h LC50 for the estuarine longnose killifish was 4.5 mg/L in seawater.

Studies on the toxicity to fish of the PGBE ester of 2,4,5-T have been reported by Kenaga (1974). This herbicide appears to be generally more toxic than the corresponding PGBE ester of 2,4-D. All rainbow trout died within 24 h at 0.13 mg/L (a.e.) of the PGBE ester of 2,4,5-T, and all fish were dead after 3 h of exposure to 0.67 mg/L of the herbicide (no water quality given). After 7 h of exposure to 0.13 mg/L of the (PGBE) Reddon^{R} formulation, all bluegill sunfish were dead. Bluegills exposed to Esteron^{R} 245 (PGBE) had 24- and 48-h LC50's of 17 mg/L in static water with a mean pH of 6.9 and a mean hardness of 29.0 mg/L. Exposures of fathead minnows to different formulations of the PGBE ester of 2,4,5-T [0.13 to 1.33 mg/L (a.e.)] all resulted in 100% mortality within 72 h (no water quality given).

Bioconcentration of the PGBE ester of 2,4-D has not been observed in fish tissues. No detectable residues of the herbicide were found in bluegill sunfish exposed to 10 mg/L PGBE ester of 2,4-D (Cope 1966).

Matida et al. (1975) noted that when a mixture of 2,4-D and 2,4,5-T as the butoxyethanol ester (commercially called "Brush Killer") was aerially spread over 9.5 hectares of forest at a rate of 150 kg/ha, no appreciable change was noted in the aquatic community. The authors were unable to detect the chemical in the stream during the 48 h observation period following spraying. Similarly, fishes (cherry salmon and dace fingerlings) showed no mortality nor abnormal behavior and the standing crop of invertebrates appeared unchanged. In a later laboratory study Matida et al. (1976) found that "Brush Killer" (mixture of 2,4-D and 2,4,5-T) exhibited toxic effects on aquatic sow bugs *Asellus hilgendorffii* and cherry salmon fry and dace fingerlings. The 96-h LC50 for the groups tested were 1.6 (sow bug), 0.6 (cherry salmon), and 1.3 (dace) mg/L. Exposures of cherry salmon fingerlings to concentrations of 0.47 and 0.62 mg/L "Brush Killer" for 96 h caused histological changes of liver parenchyma which the authors considered a nonspecific response to a toxic agent.

Experimental Results

No deaths occurred in yearling coho salmon exposed to Esteron Brush Killer for 96 h at ≤800 µg/L (nominal concentration under static conditions) nor in the flow-through exposure tanks (210 µg/L maximum nominal concentration). No deaths were observed in the subsequent seawater challenge tests (Table 12). In both the static and flow-through systems the measured amount of Brush Killer was very low even though extra care was taken in the mixing of the toxicant solutions. The reason for the low recovery is unknown.

TABLE 12. SURVIVAL OF YEARLING COHO SALMON EXPOSED TO ESTERON BRUSH KILLER AND SUBSEQUENT SURVIVAL FOLLOWING TRANSFER TO SEAWATER (MAY 31-JUNE 16, 1977).

Concentration (µg/L) nominal	measured	Percent survival[a] (96-h exposure FW)	Percent survival (290-h SW)
A. Static exposures			
Control	3.6- 4.0	100	100 (15)[a]
1	1.9- 2.6	100	100 (20)
7	2.4- 2.8	100	100 (15)
25	7.0- 7.2	100	100 (20)
75	30.7- 35.4	100	100 (15)
150	45.0- 51.0	100	100 (20)
300	72.6- 73.6	100	100 (15)
450	104.1-111.6	100	100 (10)
800	147.7-159.0	100	100 (10)
B. Flow-through exposures			
Control	0	100	100 (20)
35	23.7	100	100 (20)
70	41.4- 45.7	100	100 (20)
140	59.6- 74.0	100	100 (20)
210	84.3- 88.3	100	100 (20)

[a] *Twenty fish per concentration exposed in static test and 40 fish per concentration in flow through.*

Histological examination of coho salmon exposed to 800 µg/L Esteron (nominal concentration) for 96 h indicated no apparent affect on either liver or kidney tissue. The gills, however, showed the following abnormalities: curved gill filaments, epithelium frequently hypertrophied, and aneurisms in some lamellae (Appendix II).

Steelhead trout fry were also exposed to Esteron Brush Killer to determine if younger age groups were more susceptible than yearling fish.

In the first exposure test approximately 50% of the fry exposed to 1200 µg/L (nominal concentration) for 48 h died (Table 13 A), whereas in a test 2 weeks later, only a single death was recorded at 1200 µg/L even though the test was run for 96 h (Table 13 B). Unfortunately the water samples from the first 800 and 1200 µg/L groups were lost during extraction. Thus we do not know if total concentration of the herbicide were similar between the two tests. As was noted in the test with yearling coho salmon, the measured concentration of Brush Killer was only a fraction of the expected. Reasons for the discrepancy in both the nominal and measured concentrations of the herbicide and deaths observed between the two tests are unknown.

TABLE 13. SURVIVAL OF BIG CREEK WINTER STEELHEAD TROUT FRY EXPOSED TO ESTERON BRUSH KILLER, FAIRPLAY LABORATORY, OSU (MAY 31-JUNE 4, 1977).

Concentration (µg/L) nominal	measured	Exposure time-h[a]	Percent survival (range)
A. May 31-June 4, 1977			
Control	32.9-34.0	96 (60)	100
1	12.1-12.8	96 (60)	100
7	13.2-13.5	96 (60)	100
25	17.7-17.9	96 (60)	98.3 (100-96.7)
75	35.2-37.2	96 (60)	100
150	43.9-44.3	96 (60)	100
450	77.4-80.1	96 (60)	100
800	[b]	48 (60)[c]	88.3 (100-76.7)
1200	[b]	48 (63)[c]	50.8 (80-24.2)
B. June 12-16, 1977			
Control	6.5- 7.2	96 (60)	100
800	202.0-269.0	96 (60)	98.4 (100-96.7)
1000	240.0-243.0	96 (60)	98.4 (100-96.7)
1200	226.0-246.0	96 (60)	98.4 (100-96.7)

[a] *Total number of fish exposed in replicated tanks under static conditions.*
[b] *Samples lost during extraction.*
[c] *Tanks stocked June 2, 1977 in the morning; toxicant exposure initiated in afternoon of same day.*

Dr. D. Woodward, U. S. Fish and Wildlife Service, Fish-Pesticide Research Unit (personal communication) found 96-h LC50 values of 600-1000 µg/L with the PGBE ester of 2,4-D for cutthroat trout fry. He did not find toxicity to be affected by pH or water hardness. In a recent publication Woodward and Mayer (1978) noted that survival of cutthroat trout alevins was significantly reduced when they were continuously exposed to concentrations of 60 and 124 µg/L 2,4-D PGBE ester. The authors suggest the no-effect concentration for cutthroat trout to be 31 µg/L 2,4-D PGBE ester.

Following a simulated field application Woodward and Mayer (1978) recommended that water residues of the ester should not exceed 100 µg/L after a single application nor 31 µg/L following multiple applications.

Histological examination of the steelhead trout fry showed liver and kidney tissues to be normal, but gill tissue (including controls) showed evidence of hypertrophy of the chloride cells (Appendix II).

Examination of steelhead trout fry showed evidence of 2,4-D and 2,4,5-T residues following exposure to Brush Killer (Table A-1). The whole body residues generally increased with increasing concentration and exposure time. Fish that were moribund or dead exhibited the highest body residues (Table A-1). Fish placed in clean water for 48 h following 48 h of toxicant exposure showed slightly reduced residue levels.

TORDON 22 K (PICLORAM)

Review of Literature: Toxicity to Fish and Behavior in the Environment

Tordon^{R} (4-amino-3,5,6-trichloropicolinic acid) or picloram is one of the most potent and effective plant growth regulators currently employed (Foy 1976). There are three basic formulations: potassium salt, triisopropanolamine salt, and isooctyl ester. Tordon 22K contains 21.5% picloram (a.e.) as the potassium salt.

Picloram is effective against most perennial, broadleaf, herbacious weeds and many woody species (Klingman and Ashton 1975). It is routinely used along right-of-ways (utility lines, pipelines, highways, railways) and on pastureland, rangeland, and forests, where it is applied at rates ranging from 1/8 to 2 lb/acre (a.i.).

The effectiveness of the picloram herbicides has been directly correlated with their inherent potency, translocatability and comparative resistence to degradation (Foy 1976). Goring et al. (1965) reported that losses of picloram in the soil ranged from 58 to 96% within 1 year after application, and 78-100% within 2 years. Insignificant amounts of picloram are lost through volatilization. Picloram has been shown to undergo extensive photodegradation by ultraviolet light and sunlight in aqueous solutions or soil surfaces (Hall et al. 1968).

Although degradation of picloram is slow in temperate climates (National Research Council Canada 1974, NRCC), decomposition in the soil does occur by nonbiological processes (Hance 1969). Adsorption to soil colloids is minimal in neutral or alkaline sandy loam soil, but increases with decreasing pH, increasing organic content, and increasing concentrations of hydrated iron and aluminium oxides (Foy 1976).

Tordon 22K is highly soluble in water (40% w/w). Movement of the herbicide in the soil profile is governed by the net water flow, with maximum losses occurring under warm, humid conditions, following heavy rainfall, and in light soils low in organic content. The removal of picloram through rainfall and leaching is one of the major factors governing

its dissipation under field conditions (NRCC 1974). This mobility is also of environmental concern, as leached picloram may be transported to aquatic ecosystems such as ponds, lakes, and streams. Residue levels in surface runoff have reached 2 mg/L following application at 1.1 kg/ha (NRCC 1974). However, studies have indicated that under most conditions only small proportions of picloram (less than 5%) applied to a watershed are transported in surface runoff.

Only negligible residues of picloram occur in streams, apparently due to rapid dilution of the herbicide (Haas et al. 1971). Field plots adjacent to the mouth of a small stream were treated with 1.1 kg/ha of picloram, and water samples were collected 0, 0.8, and 1.6 km downstream from the plots following each rain for 5 months after application. Picloram was detected in the stream samples only during the first significant runoff (0.029 mg/L). No residues were found in subsequent samples (Haas et al. 1971).

Picloram contamination in lakes has not been reported, but levels in farm ponds adjacent to plots treated with 1.1 kg/ha picloram reached 1 mg/L (NRCC 1974). Dissipation of the herbicide in ponds has been shown to be rapid. One study found an initial decline of 14 to 18% of picloram per day, followed by a decline of less than 1% per day 15 weeks after application (Haas et al. 1971). Residues of picloram in the pond-bottom sediments (148 µg/kg) immediately following application were only twice that in the water (Kenaga 1973, in NRCC 1974). After 75 days, residues of 7 µg/kg picloram were detected in the pond-bottom sediments and 0.1 µg/kg picloram was found in the water.

It is apparent from a number of studies that the toxicity of picloram to fish is influenced by its formulation and the water quality (NRCC 1974, Woodward 1976, Sergeant et al. 1970). Technical grade picloram (a.i. 90%) was found to be more toxic under alkaline conditions (Woodward 1976). Increasing the pH from 6.5 to 8.5 increased the toxicity to cutthroat and lake trout by a factor of 2 in both species. Increasing temperature did, but increasing hardness did not, lead to an increase in toxicity (Woodward 1976).

The acute toxicity of picloram varied considerably with the formulation and fish species. The isoocytl ester of picloram appears to be the most toxic commercial formulation (NRCC 1974, Sergeant et al. 1970, Kenaga 1969). LC50's reported for this formulation are approximately 1 mg/L for sensitive species. Toxicity levels of Tordon 22K (potassium salt) are considerably lower as shown for several fish species in Table 14.

Based on available information, chronic picloram toxicity to fish is not cumulative in terms of lethality (Woodward 1976, NRCC 1974). Longterm exposures, however, have been shown to affect fish development and growth (Woodward 1976), and swimming response and liver histopathology (Sergeant et al. 1970). Woodward (1976) observed that the no-effect concentration of technical grade picloram for lake trout was apparently <35 µg/L, as this level of herbicide reduced fry survival and growth. Most mortalities occurred during yolk absorption, which took 4-5 days longer in picloram-treated fish.

TABLE 14. MORTALITY DATA FOR SEVERAL FISH SPECIES EXPOSED TO TORDON 22K FOR 96 HOURS[a].

Fish species	Water temperature (°F)	Concentration (mg/L) a.e.	Percent mortality
Black bullhead	50	91[b]	50
		69[c]	0
Bluegill	65	5.4	50
Brook trout	50	91	50
		69	0
Brown trout	50	52	50
		22	0
Fathead minnows	50	29	50
		22	0
Green sunfish	50	91	50
		39	0
Lake emerald shiner	69-78	30	50
Rainbow trout	50	58	50
		22	0

[a] *Modified from Kenaga (1969).*
[b] *Calculated or derived 96-h LC50 values.*
[c] *Highest concentration producing no mortality.*

Green sunfish exposed to the 99% analytical grade picloram (1.2 ppm, a.e.) were not affected whereas the technical grade or the 22% commercial formulation of picloram (for up to 1 hour) caused immobilization but not death (Sergeant et al. 1970). Recovery of normal swimming response followed transfer to clean pond water. Two subsequent exposures to the herbicide shortened the recovery times; however, after a fourth exposure, many of the fish failed to recover. Analytical grade picloram did not affect green sunfish swimming behavior. Sergeant and coworkers suggested that technical grade and commercial formulations of picloram might contain a toxic impurity.

Electron micrographs of hepatocytes from green sunfish exposed to technical picloram (1.2 mg/L) for 1 day revealed abnormalities in these cells (Sergeant et al. 1970). These ultrastructural changes involved the disappearance of rough-surfaced endoplasmic reticulum and an increase in tubular or smooth endoplasmic reticulum. These changes were also accompanied by liver enlargement. Increasing the exposure time of the herbicide from 1 to 5 days did not alter the ultrastructural pattern.

Residue analyses of aquatic organisms exposed tp picloram indicate that this herbicide is not bioconcentrated in invertebrates or along food chains (NRCC 1974). Daphnia exposed to 1 mg/L of the potassium salt of picloram had whole body residues of the herbicides equal to that present in the water (Hardy 1966). Bioconcentration of picloram (acid) was not evident in mosquito fish exposed to 1 mg/L (a.e.) for 18 days (Youngson and Meikle 1972). The concentration factor for these fish on a wet weight, whole body basis was only 0.02. The 18 days of exposure to picloram was adequate to achieve a steady state level of accumulation in the mosquitofish.

Experimental Results

In our study, the 24-h LC50 of Tordon 22K was estimated to be 17.5 mg/L. There was no apparent effect of picloram on the (Na,K)-stimulated ATPase activity of the gills (Table 15). Histological examination of fish exposed to 5 mg/L Tordon 22K for 144 h revealed abnormal liver and gill tissues (Appendix II). When the survivors of the Tordon 22K exposure were placed in sea water, a 25% mortality occurred in the group previously exposed to 0.25 mg/L (Fig. 6). We do not know the reason for these deaths in seawater after exposure to this low Tordon 22K concentration. Similar seawater mortalities at low concentrations occurred following the Tordon 101 and Dicamba exposures, and they also were not explainable--different tanks were used for seawater challenge tests for the three chemicals and oxygen concentrations were @9 mg/L when the mortalities occurred.

TABLE 15. SURVIVAL AND GILL ATPASE OF YEARLING COHO SALMON EXPOSED TO TORDON 22K IN FRESHWATER AND SUBSEQUENT SURVIVAL FOLLOWING TRANSFER TO SEAWATER (DEC. 9-27, 1976).

Concentration (mg/L)		Percent survival[a]	Gill ATPase[b]	Percent survival
nominal	measured	(144-h exposure FW)		(288-h SW)
Control	0-0.12	100	2.5	100
0.25	1.67	100	NT[c]	75
0.50	1.89	100	NT	100
0.75	2.76	100	NT	100
1.0	3.89-4.23	100	2.2	100
5.0	10.54-11.84	100	1.8	100
15.0	31.81-41.23	0[d]		NT
30.0	37.95-45.10	0[e]		NT

[a] *Twenty fish exposed per concentration.*
[b] *(Na,K)-stimulated ATPase activity; mean of 4 fish.*
[c] *Not measured.*
[d] *All dead in 48 h.*
[e] *All dead in less than 8 h.*

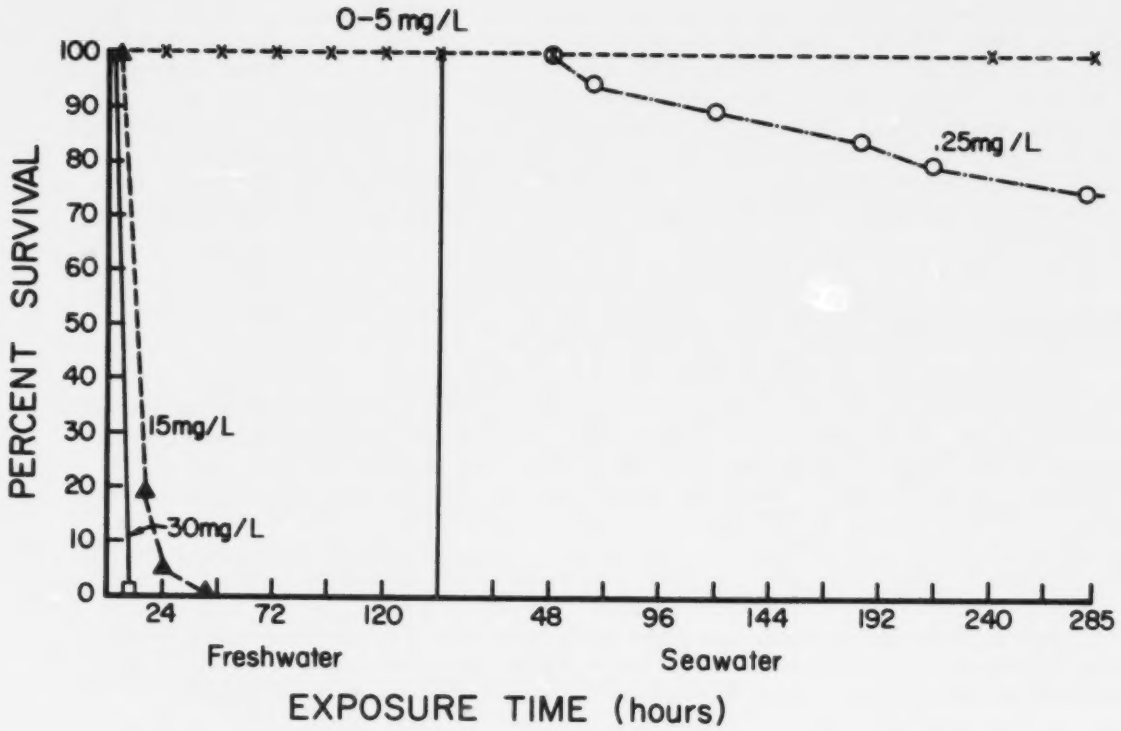

Figure 6. Percent survival of yearling coho salmon during exposure to Tordon 22K (picloram) in freshwater and subsequent survival upon transfer to seawater.

An experiment was designed to test fish density and ammonium concentration as possible factors relating to the observed seawater mortality following exposure to low concentrations of Tordon. Following exposure to the toxicant, survivors were placed in seawater and survival monitored. No apparent ill effects or deaths were observed in seawater tests of the various fish density and ammonia groups (Table 16). Seawater deaths, therefore, did not appear to be involved with fish density, or ammonia levels during toxicant exposure.

TABLE 16. SURVIVAL OF YEARLING COHO SALMON AT THREE FISH DENSITIES EXPOSED TO TORDON 22K OR TORDON 22K PLUS AMMONIUM CHLORIDE IN FRESHWATER, AND THE SUBSEQUENT SURVIVAL FOLLOWING TRANSFER TO SEAWATER (FEB. 11-28, 1977).

Fish density No./tank	Concentration (mg/L)				Percent survival	
	Nominal Tordon	Measured	NH_4Cl (added)	NH_3-N[a/]	144-h exposure FW	260-h SW
10	Control	-	0	0.34	100	100
20	Control	-	0	0.70	100	100
5	Control	-	0.80	1.05	100	100
10	0.05	0.058	0	0.25	100	100
20	0.05	0.048	0	0.64	100	100
10	0.10	0.11	0	0.38	100	100
20	0.10	0.11	0	0.63	100	100
10	0.25	0.50	0	0.23	100	100
20	0.25	0.42	0	0.65	100	100
5	0.25	0.32	0.80	1.01	100	100
10	0.50	0.59	0	0.22	100	100
20	0.50	0.71	0	0.61	100	100
5	0.50	0.67	0.80	1.04	100	100
10	0.80	0.89	0	0.27	100	100

[a/] *Measured ammonia level, average of six daily values.*

TORDON 101

Review of Literature: Toxicity to Fish and Behavior in the Environment

The Dow Chemical Company's Tordon[R] 101 formulation contains 5.7% a.e. (0.54 lb/gal) triisopropanolamine salt of picloram plus 21.2% a.e. (2.0 lb/gal) 2,4-D as the triisopropanolamine salt. The interaction of picloram with 2,4-D enhances the translocation of 2,4-D in plants (Foy 1976). Other reported interactions are either adaptive or competitive. Tordon 101 is used in the control of annual and perennial weeds, woody plants, and vines on non-crop lands including right-of-ways. Label specifications suggest Tordon 101 application at the rate of 1/2 to 3 gal/acre for the control of broadleaf weeds, and 1 to 4 gal/acre for woody plants and vines.

The effect of Tordon 101 on fish has been reviewed by Kenaga (1969). Salmonids were exposed to varying levels of the toxicant in Lake Huron water at 10°C. Lynn (1965 in Kenaga 1969) reported the 96-h LC50's for brook, brown, and rainbow trout were 50.9, 48.8, and 31.8 mg/L (a.e.) 2,4-D, and 13.7, 13.1 and 8.6 mg/L (a.e.) picloram, respectively. Fathead minnows were considerably more sensitive to Tordon 101. The 96-h LC50 for this species (10°C) was 13.7 mg/L 2,4-(a.e.) and 3.7 mg/L (a.e.) picloram.

Experimental Results

Static exposure --

In our study, a static toxicity test in December indicated a 24-h LC50 of Tordon 101 to be 20 mg/L (concentration of 2,4-D and picloram combined), and the toxicant appears to have a steep mortality curve (Fig. 7). Tordon 101 appeared to cause a slight reduction in the (Na,K)-stimulated ATPase activity of the gills, but this lowered activity did not appear to affect seawater survival (Table 17). The reduced survival during the seawater challenge of yearling coho salmon previously exposed to 0.25 and 0.50 mg/L is an interesting but unexplained phenomenon (Fig. 7). A number of the dead and dying fish were examined for disease or parasites to determine if this could possibly account for the deaths, but no abnormal conditions were noted.

TABLE 17. SURVIVAL AND GILL ATPASE OF YEARLING COHO SALMON EXPOSED TO TORDON 101 IN FRESHWATER AND THE SUBSEQUENT SURVIVAL FOLLOWING TRANSFER TO SEAWATER (NOV. 30-DEC. 21, 1976).

Concentration (mg/L)		Percent survival[a] (144-h exposure FW)	Gill ATPase[b]	Percent survival (360-h SW)	Gill ATPase[c]
nominal	measured				
Control	-	100	1.9	100	4.0
0.25	0.29	100	NT[d]	25	4.9
0.50	0.50- 0.62	100	NT	65	
1.0	1.32- 1.59	100	1.4	100	5.0
5.0	6.42- 7.35	100	1.5	100	4.0
15.0	18.1- 19.8	100	0.9	100	
30.0	31.5	0[e]		-	
60.0	67.8	0[f]		-	

[a] *Twenty fish exposed per concentration.*
[b] *(Na,K)-stimulated ATPase activity; mean of 4 fish (freshwater).*
[c] *Mean of 5 fish (seawater).*
[d] *Not measured.*
[e] *All fish dead within 60 h.*
[f] *All fish dead within 2.5 h.*

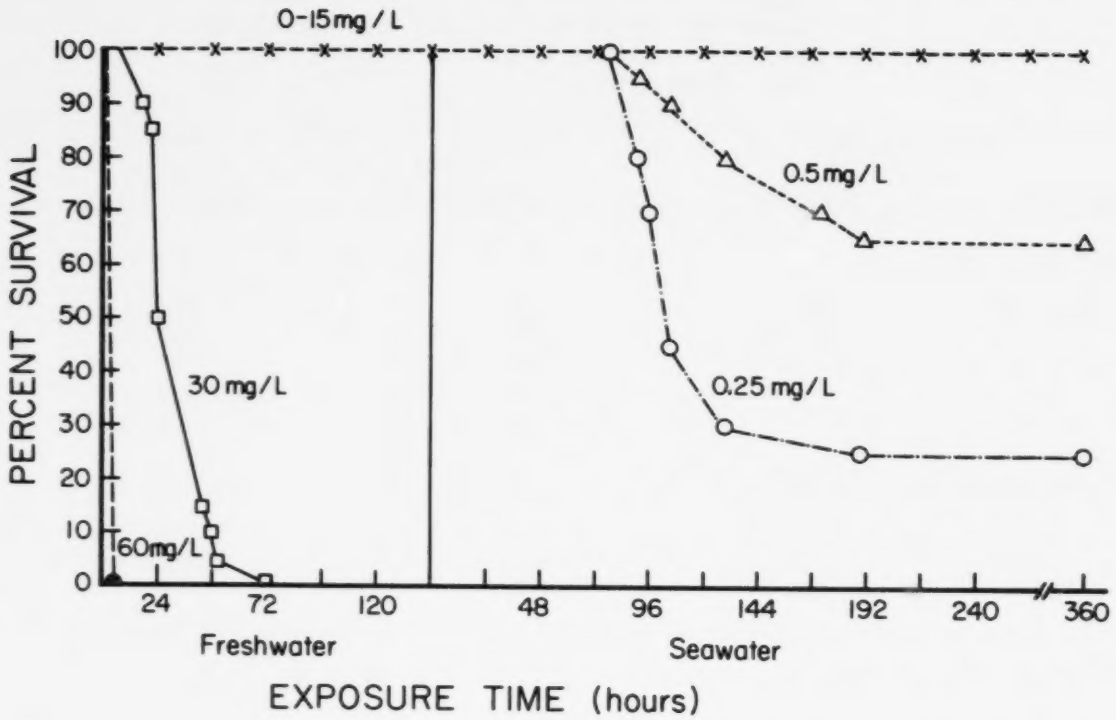

Figure 7. Percent survival of yearling coho salmon during exposure to Tordon 101 (2,4-D + picloram) in freshwater and subsequent survival upon transfer to seawater.

Another test with Tordon 101 was initiated in mid-March. Half of the test tanks were dosed with toxicant stock that was mixed fresh daily while the other tanks were dosed with toxicant stock that was prepared when the experiment was initiated. There appeared to be no difference in toxicant effect regardless of when the toxicant stock was mixed (Table 18). The highest toxicant exposure concentration (22 mg/L) used in the March test was close to the estimated 24-h LC50 of December (20 mg/L), however, 95% of the yearling coho salmon exposed to 22 mg/L Tordon 101 (nominal concentration) died. The reason for the poor recovery of Tordon 101 is unknown, but if the measured values are real, then yearling coho salmon are more sensitive as smolts than as non-smolts. There was no mortality noted during the seawater challenge of fish from groups representing either of the methods used in toxicant preparation, except the sole survivor of 22 mg/L Tordon 101 died in the first 24 h of the seawater exposure (Table 18).

TABLE 18. SURVIVAL OF YEARLING COHO SALMON EXPOSED TO TORDON 101 IN FRESHWATER, AND THE SUBSEQUENT SURVIVAL FOLLOWING TRANSFER TO SEAWATER (MAR. 13-29, 1977).

Concentration (mg/L) nominal	measured	Percent survival[a/] (144-exposure FW)	Percent survival (240-h SW)
New stock daily			
Control	0	100	100
0.25	0.13-0.16	100	100
0.50	0.12-0.13	100	100
1.0	-	100	100
22.0	8.41-8.83	5	0
Stock mixed Mar. 13, 1977			
0.25	0.19-0.38	100	100
0.50	0.41-0.50	100	100
22.0	9.21-9.7	0	-

[a/] *Twenty fish exposed per concentration.*

Flow-through exposure--

Following marking (fin excision) and time for acclimatization to new surroundings, yearling coho salmon were exposed to Tordon 101 in the flow-through system beginning on Mar. 28, 1977. Groups of yearling coho salmon were placed in seawater following 144-h and 380-h exposure to the four Tordon 101 concentrations. No deaths occurred in either the fish exposed to Tordon 101 or the control fish during the 240 h of seawater exposure (Table A-2). The (Na,K)-stimulated ATPase activity was not affected by any of the Tordon 101 concentrations tested (Table A-2). Exposure to Tordon 101 for 360 h produced no apparent effect on the condition factor of yearling coho salmon (Table A-3). The fish all fed well during the exposure, and the general decline in condition factor was probably the normal change in the length-weight relationship that occurs as the fish undergo transformation to smolts (Wagner 1974a).

A release of 10 groups of coho salmon exposed to Tordon 101 for 360 h and 5 groups exposed for 96 h was made into Crooked Creek on April 13, 1977, and downstream movement was monitored. The coho salmon exposed to 0.3 mg/L for 15 days showed slightly stimulated migration compared to the controls, whereas the other concentrations (0.6, 1.2, and 1.8 mg/L) showed a dose-dependent inhibitory response. Approximately a 10% difference in movement was noted between controls and the group receiving 1.8 mg/L Tordon 101 over the 84 days that migration was monitored (Fig. 8a). The differences observed, however, were not statistically significant ($P = 0.05$).

Coho salmon that received 96 h exposure to Tordon 101 (0.3-1.8 mg/L) prior to release did not migrate as well as the control group except for the 0.3 mg/L group which again showed slight migratory stimulation. In the other 96-h exposure groups (0.6, 1.2, and 1.8 mg/L) the migratory response was less than observed for the control but no relationship to concentration of Tordon 101 was apparent (Fig. 8b). The majority of downstream movement of all groups of coho salmon occurred within the first 5 days of release (Table A-4).

Histological examination of gill, liver, and kidney tissue of coho salmon from the various chronic (15-day) Tordon 101 exposures showed only peribiliary necrosis in the liver that was not present in the controls (Appendix II). The consequences of this type of tissue degeneration or the effect on the fish in terms of ultimate survival is unknown.

Coho salmon that had been exposed for 15 days to 1.8 mg/L Tordon 101 and a comparable group of control fish were killed and several tissue and organ systems excised and analyzed for bioconcentration of Tordon 101. No apparent accumulation of 2,4-D or picloram was noted. Youngson and Meikle (1972), however, did find residues of 0.21 mg/kg in mosquitofish exposed to 1 mg/L picloram for 18 days.

DINOSEB (PREMERGE)

Review of Literature: Toxicity to Fish and Behavior in the Environment

Dinitrophenols are the oldest organic chemicals patented for selective weed control (Kaufman 1976). Two of these compounds, dinoseb (2-sec-butyl-4, 6-dinitrophenol) and dinosam have become widely used herbicides. The phenol form of dinoseb is oil soluble and formulated as an emulsifiable concentrate. The amine or ammonium salts of dinoseb are water soluble and are formulated under a variety of trade names. Dow Premerge (alkanol amine salt) was utilized in this study. The general use of dinitroamines and ammonium salts has been for postemergent control of most seedling weeds and grasses in cereal, tree, and vegetable crops. Application rate of the amine salt of dinoseb is normally from 1.5 to 6 lb/acre (a.i.) depending upon the crop. Dinoseb is also applied in some areas to control ditch bank weeds (Woodward 1976).

The fate and behavior of herbicides is generally dependent upon their rate of decomposition, volatilization, movement, uptake and absorption (Kaufman 1976). Dinitrophenols appear to be stable in acid solutions, but undergo photolysis at alkaline pH. Volatilization or codistillation of dinoseb has been shown to occur under conditions of soil acidity, high temperature, and surface soil moisture. Heavy rains following application may cause leaching, particularly in alkaline soils; although in mineral clays, adsorption may slow the rate of leaching (Montgomery et al. 1976, Kaufman 1976). Certain soil microorganisms readily degrade dinitrophenols. The residual life of dinoseb under warm, moist soil conditions is 3-5 weeks; residual carry-over is not expected to occur from one season to the next (Klingman and Ashton 1975).

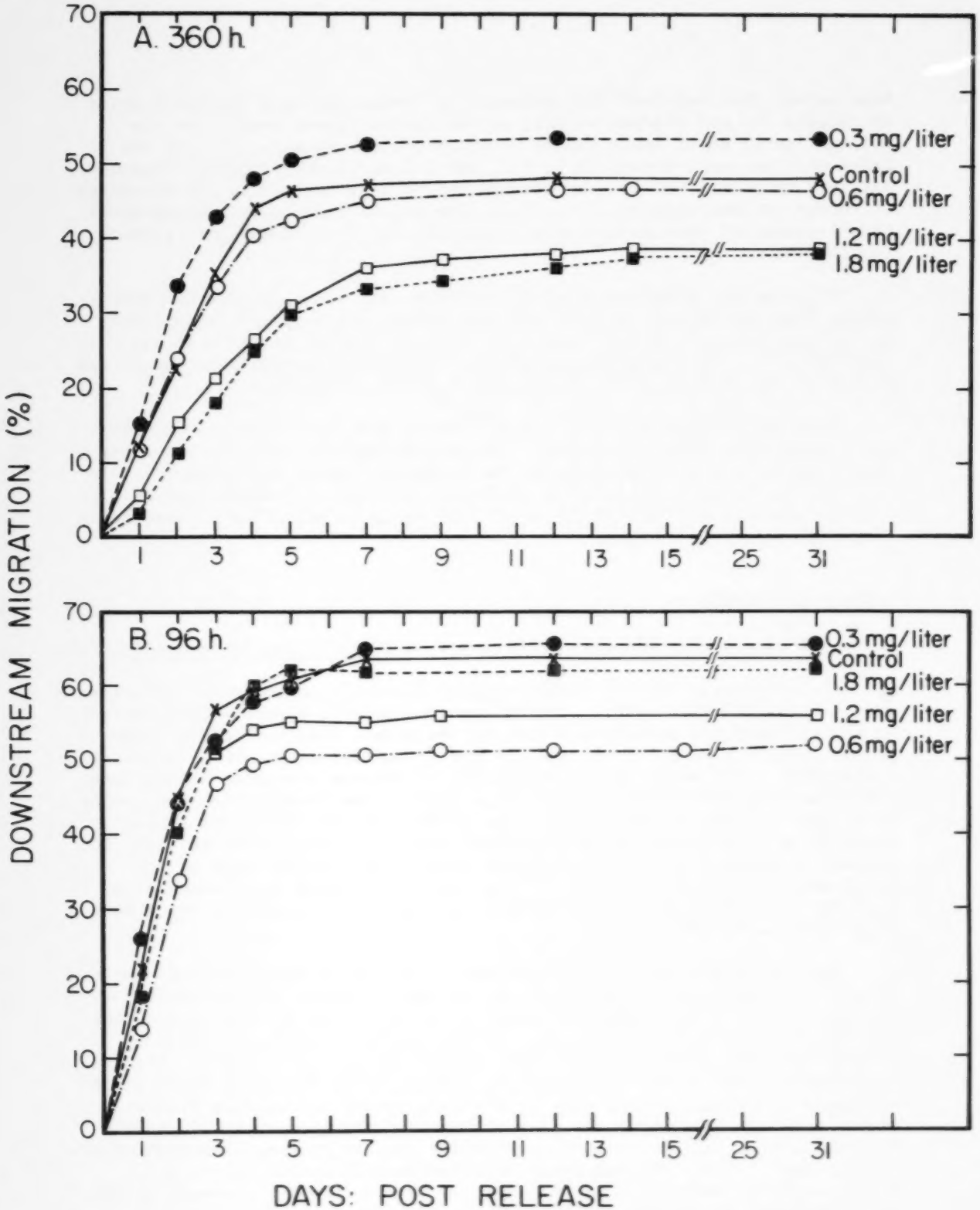

Figure 8. Percent downstream migration of yearling coho salmon following exposure to Tordon 101. A. represents 209-213 coho salmon released per group following 360 h of Tordon 101 exposure. B. represents 107-115 coho salmon released per group following 96 h of Tordon 101 exposure.

Dinoseb has a high toxicity to man, animals, and fish. The acute and chronic effects of dinoseb on cutthroat and lake trout was investigated by Woodward (1976), who found that toxicity was largely dependent upon water quality. Decreasing the water pH resulted in a corresponding increase in dinoseb toxicity to fish. Similar findings were reported by Lipschuetz and Cooper (1961) for technical grade dinoseb. Decreasing the pH from 8.0 to 6.9 increased the toxicity of dinoseb to rainbow trout by a factor of 5. High water temperature and high hardness also tend to maximize the toxicity of dinoseb to fish, but to a lesser extent than pH (Webb 1951, Woodward 1976).

The 96-h LC50's, under varying conditions of pH, temperature, and hardness, ranged from 0.41 to 1.35 mg/L dinoseb for cutthroat trout and from 0.032 to 1.40 mg/L for lake trout (Woodward 1976). Lipschuetz and Cooper (1961) observed similar dinoseb toxicity values for rainbow trout. Their 24-h LC50 at pH 8.0 was 0.30 mg/L dinoseb (tech.), and at pH 6.9 was 0.073 mg/L (18°C). Western blacknose dace *(Rhinichthys atratulus)* in water of pH 8.0 and a temperature of 21°C had a 24-h LC50 of 0.24 mg/L dinoseb (tech.). Using a dinoseb formulation composed of secondary butyl dinitrophenol and secondary amylbutyl dinitrophenol, Webb (1951) reported a 24-h LC50 for redside shiners *(Richardsonius balteatus hydroflux)* of 0.16 mg/L in soft water (18 mg/L hardness, pH 7.6) and of 0.24 mg/L in hard water (105 mg/L hardness, pH 8.2).

Woodward (1976) did not observe cumulative mortality of lake and cutthroat trout with chronic exposures (8 to 12 days) to dinoseb. However prolonged exposures to 0.005 to 0.010 mg/L dinoseb affected yolk absorption time and fry growth. Yolk absorption time increased by 6 to 9 days over that of the controls, and fry growth was reduced at all concentrations of dinoseb tested.

Experimental Results

Static exposure--

In our study, Dow Premerge was highly toxic to yearling coho salmon during the static freshwater exposures. A sudden and sharp rise in mortality occurred between 75 µg/L (no deaths) and 100 µg/L (90% mortality in 144 h) as noted in Fig. 9. The 24-h LC50 was calculated at 190 µg/L while the 144-h LC50 was estimated to be 88 µg/L. Nominal concentrations were used to calculate the LC50 values since the measured concentrations (combined aliquots from daily samples) were extremely variable and often only a fraction of the expected (Table 19). Coho salmon exposed to 300 and 500 µg/L dinoseb were all dead within 24 h, with 40-45% dying during the first 30 minutes of exposure (Fig. 9). When the surviving fish (control to 100 µg/L dinoseb) were transferred to seawater, all fish survived, even those that appeared weakened from the prior toxicant exposure (Table 19). No apparent dinoseb effect was noted on the (Na,K)-stimulated ATPase activity of the gills (Table 19).

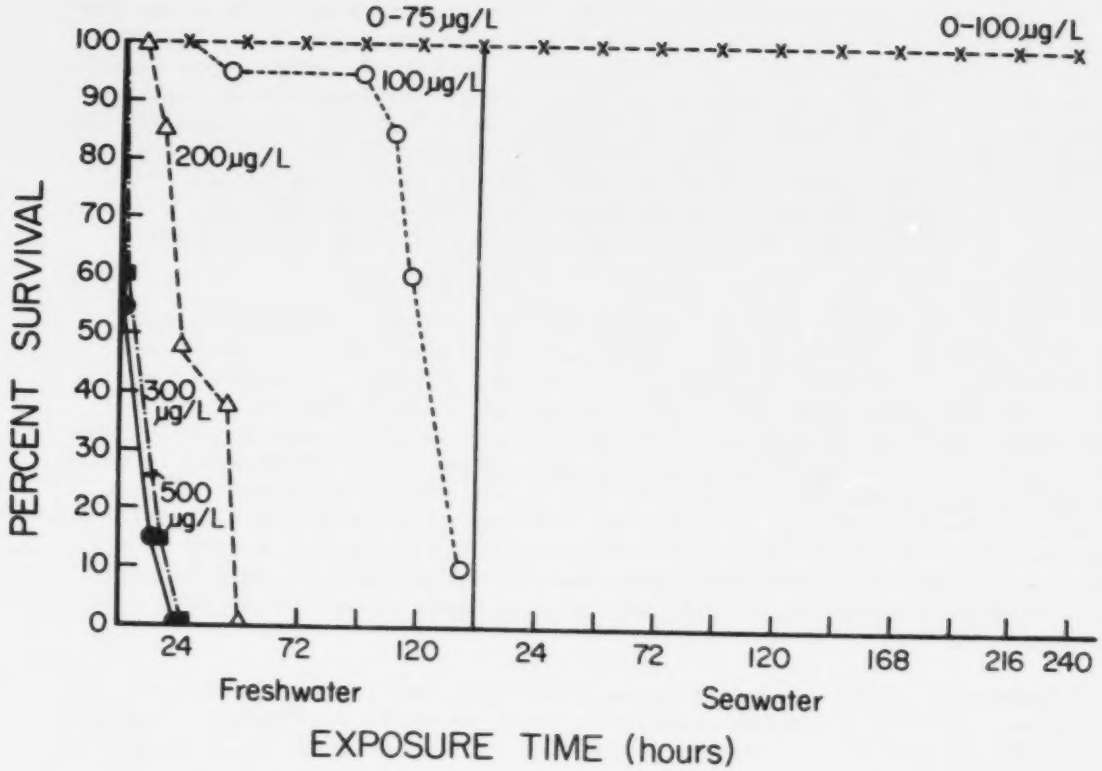

Figure 9. Percent survival of yearling coho salmon during exposure to dinoseb (Dow Premerge) in freshwater and subsequent survival upon transfer to seawater.

TABLE 19. SURVIVAL AND GILL ATPASE OF YEARLING COHO SALMON EXPOSED TO DINOSEB IN FRESHWATER AND SUBSEQUENT SURVIVAL FOLLOWING TRANSFER TO SEAWATER (FEB. 22-MAR. 10, 1977).

Concentration (µg/L) nominal	measured	Percent survival[a/] (144-h exposure FW)	Gill ATPase[b/]	Percent survival (260-h SW)
Control	0	100	2.9	100
25	1.1- 12.0	100	2.7	100
50	3.0- 11.8	100	2.8	100
75	3.1- 13.1	100	3.7	100
100	16.6- 61.7	10	NT[c/]	100
200	23.0- 65.4	0	-	-
300	141.0-209.0	0	-	-
500	241.0-275.2	0	-	-

[a/] *Twenty fish exposed per concentration.*
[b/] *(Na,K)-stimulated ATPase activity; mean of 4 fish.*
[c/] *Not measured.*

Flow-through exposure--

As dinoseb was found to be extremely toxic to coho salmon and had the potential for interacting with anadromous salmon because of its use in weed control along ditches and irrigation canals, it was chosen for study in the flow-through system. Following marking (fin excision) and a 5-day acclimatization period, yearling coho salmon were exposed to dinoseb (Dow-Premerge) beginning April 19, 1977. Two days after initiation of the exposure, the fish in the highest concentration (60 µg/L) began to turn dark, eat poorly, and die. The deaths occurred at concentrations considerably lower than had previously caused death under static conditions in late February. Whether the smolting of the fish, the use of a flow-through system or a combination of both was responsible for the greater toxicity is unknown. During the first 6 days of exposure to dinoseb, 93% mortality occurred in the groups receiving 60 µg/L and 8% mortality was noted in the 40-µg/L group. Two deaths occurred during the sea water challenge in the group originally exposed to 60 µg/L dinoseb for 144 h (Table 20). Considering the poor physical appearance of the 40 and 60 µg/L exposed groups prior to placing in salt water, it was surprising that there were so few deaths (Table 20). The majority of dark colored fish regained their silvery coloration during the 10-day seawater challenge. There were no mortalities during either toxicant exposure or seawater challenge of the coho salmon exposed to 10 or 20 µg/L dinoseb for 16 days. However, all the fish exposed to 60 µg/L and almost all exposed to 40 µg/L died during the 16 days of exposure (Table 20). The gill (Na,K)-stimulated ATPase activity was unaffected after 6 or 16 day exposures to dinoseb (Table 20).

TABLE 20. SURVIVAL OF YEARLING COHO SALMON EXPOSED TO DINOSEB IN FRESHWATER AND SUBSEQUENT SURVIVAL FOLLOWING TRANSFER TO SEAWATER (APR. 19-MAY 16, 1977).

Concentration (µg/L) nominal	measured	Percent survival[a]	Gill ATPase[b]	Percent survival[a] (240-h SW)
A. 144-h exposure				
Control	0.5	100 (270)	6.93 (10)	100.0 (20)
10	7.4- 7.9	100 (250)	NT[c]	100.0 (20)
20	15.3-17.3	100 (250)	6.54 (10)	100.0 (20)
40	38.6	92 (300)	5.36 (10)	100.0 (20)
60	54.5-58.3	7 (339)		90.0 (20)
B. 384-h exposure				
Control	0.5	100 (270)	6.81 (20)	100.0 (30)
10	6.2- 8.8	100 (250)	NT	100.0 (30)
20	15.3-17.3	100 (250)	6.43 (20)	100.0 (39)
40	30.7-38.6	6 (300)[d]	NT	NT
60	52.9-60.0	0 (339)[e]		

[a] *Number of fish exposed in parentheses.*
[b] *(Na,K)-stimulated ATPase activity; mean with sample size in parentheses.*
[c] *Not measured.*
[d] *Terminated on day 11 to restock tanks.*
[e] *All dead within 11 d.*

A release of dinoseb-exposed coho salmon yearlings was made into Crooked Creek May 5, 1977, and the downstream migration of the various groups monitored. Coho salmon exposed to 10 µg/L dinoseb for 14 days showed slightly better migration than the controls or the 20 µg/L group, however, no significant difference in migration times were noted for the three groups (Fig. 10A). Of the yearling coho salmon exposed for 96 h, the control and 20-µg/L dinoseb treated fish showed the best migration, although the 10-and 40-µg/L groups had only 10% fewer migrants (Fig. 10B). The group exposed to 60 µg/L for 24 h also exhibited a 10% lesser migratory tendency than the controls (Fig. 10B). Fish exposed to 40 or 60 µg/L dinoseb for 48 h showed a dose-dependent inhibition in average percent downstream migration (Fig. 10C). Considerable variation in migration rates occurred, however, between replicated exposure tanks [controls, 20 and 60 µg/L (Table A-5)]; and only one of the two 60 µg/L groups (which experienced mortality during the toxicant exposure) showed statistically less migration than the controls ($P = 0.05$).

Yearling coho salmon exposed to 100 µg/L dinoseb for 114 h showed extensive necrosis of the liver, kidney, and gill lamellae (Appendix II). Histological examination of tissues of coho salmon exposed to 60 and 40 µg/L dinoseb for 168 and 336 h, respectively, showed only minor degenerative changes (Appendix II). This lack of histological damage is surprising considering that total mortality occurred at the highest concentration (60 µg/L) during 11 days of exposure to dinoseb. Tissue samples of coho salmon analyzed for dinoseb showed some present in the eight tissues examined (Table A-6). The spleen, gall bladder, liver, and kidney appeared to be the sites of major accumulation.

DIQUAT

Review of Literature: Toxicity to Fish and Behavior in the Environment

Diquat (6,7-dihydrodipyrido(1, 2·a: 2', 1-c) pyrazinediium ion) has the trade names of Ortho^{R} Diquat and Regone^{R}. It is registered for use as an aquatic herbicide in canals, lakes, and ponds, and on non-food seed crops such as alfalfa, clover, and vetch. For additional information on diquat's properties and persistence, refer to the section on paraquat.

Diquat application rates of 1.5 to 2.5 mg/L or 1 gal per surface acre have been shown to be effective in the control of aquatic vegetation in small, stillwater ponds in western Oregon. There appears to be a wide margin of safety between this rate of application and lethal levels of diquat to fish in hard water. Under laboratory conditions, Bimber et al. (1976) found that yellow perch experienced a significant level of respiratory stress at diquat concentrations comparable to those normally applied for aquatic weed control (no water quality given). Diquat is moderately toxic as shown by its acute toxicity to various fish species (Table 21). The toxicity has been shown to vary considerably depending upon hardness and pH (Surber and Pickering 1962). Wilson and Bond (1969) found that the addition of pond mud to the test aquaria changed the 96-h LC50 of diquat for the amphipod *(Hyalella azteca)* from 0.046 to 6.8 mg/L. The reduction in toxicity was probably due to the adsorption of the

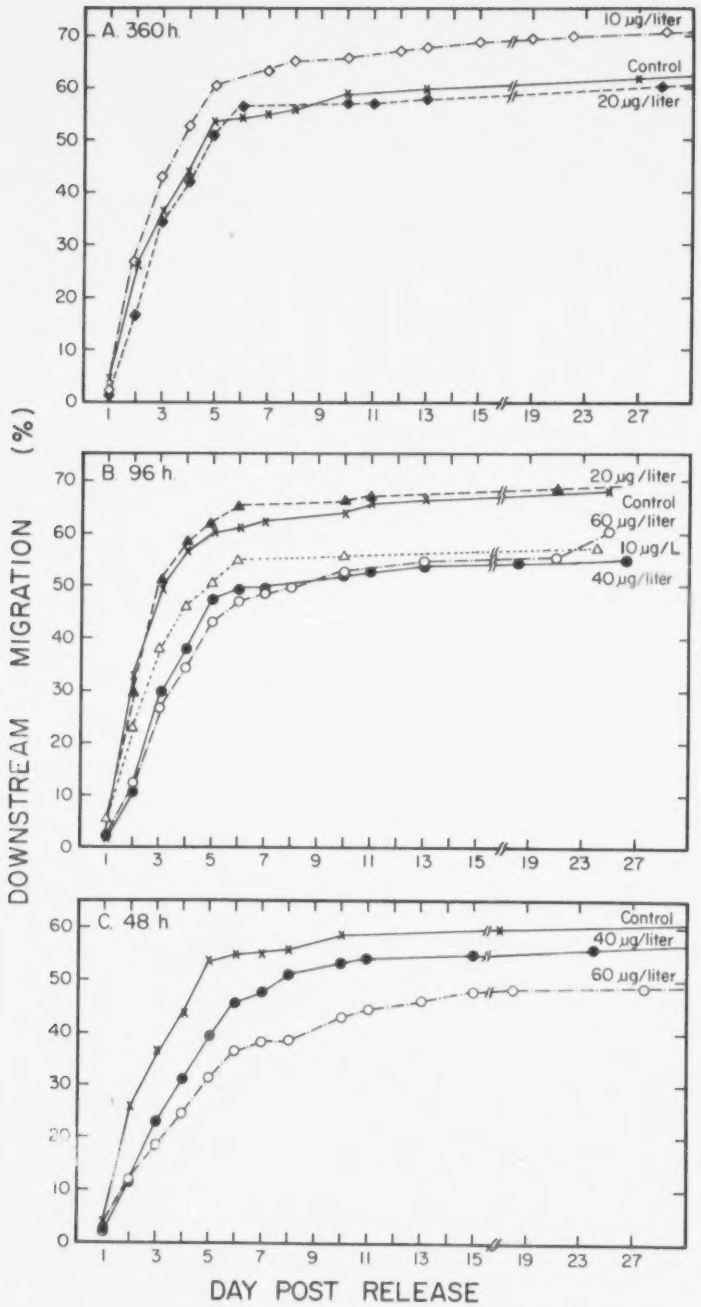

Figure 10. Percent downstream migration of yearling coho salmon following exposure to dinoseb (Dow Premerge). A. represents 203-219 coho salmon released per group following 360 h of dinoseb exposure. B. represents 98-115 coho salmon released per group following 96 h of dinoseb exposure except for the 60 µg/L group exposed for 25 h. C. represents 107 and 197 coho salmon released following 48 h exposure to 40 and 60 µg/L dinoseb, respectively.

TABLE 21. ACUTE TOXICITY OF DIQUAT TO VARIOUS FISH SPECIES[1/]

Species	Toxicity test	LC 50[2/] (mg/L)	pH	Temperature	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)	Reference
Carassius auratus (goldfish)	static, field concrete pond	35 (96 h)	8.2	48-89°F	233	290	Gilderhus (1967)
Esox lucius (northern pike)	static, field concrete pond	16 (96 h)	8.2	48-89°F	233	280	Gilderhus (1967)
Fundulus similis (killifish)	flowing, lab.	1.0 NTE (48 h)[3/,4/]					Butler (1965)
Ictalurus punctatus (channel catfish-fry)	static, lab.	10.0 NTE (111 h)[3/]	8.4	23.3-25.8°C	75	78	Jones (1962)
I. punctatus	static, lab.	10.0 NTE (96 h)[5/]		75°F			Lawrence et al. (1965)
Lepomis cyanellus (green sunfish)	static, plastic pool	4.0 NTE[3/]	8.3-8.9			120-189	Yeo (1967)
L. macrochirus (bluegill-fry)	static, lab.	10.0 NTE (12 d) 4.0 NTE (96 h) 25.0 (30 h)	8.4	22-25°C 23.3-25.8°C	75	78	Hiltibran (1967) Jones (1962)
L. macrochirus	static, lab.	19 (48 h)		24°C			Cope (1966)
L. macrochirus (fingerlings)	static lab.	525 (24 h) 150 (48 h)	6.9	25°C	40	29	Hughes and Davis (1962)
L. macrochirus	static, field concrete pond	35.0 (96 h)	8.2	48-89°F	233	280	Gilderhus (1967)
L. macrochirus	static, lab.	9-10 LD_{10} (97 h)[5/]		75°F			Lawrence et al. (1965)
L. macrochirus	static, lab.	72 (96 h) 140 (96 h)	7.4 8.1	25°C 25°C	22 312	22 341	Surber and Pickering (1962)
Micropterus dolomieui (smallmouth bass)	static, plastic pool	0.5 NTE[3/] 4.0 LC_{15} (96 h)	8.3-8.9			120-209	Yeo (1967)
	static lab.	10.0 LD_{10} (96 h)		75°F			Lawrence et al. (1965)
M. dolomieui (fry)	static, lab.	2.5 (24 h)		22-25°C			Hiltibran (1967)
M. salmoides (largemouth bass-fry)	static, lab.	1.0 NTE (96 h)		22-23°C	75	78	Jones (1962)
M. salmoides	static, lab.	7.8 (96 h)	8.3	25°C	20	21	Surber and Pickering (1962)
M. salmonides	static, lab.	11.0 (48 h)					Muirhead-Thompson (1971)
Morone saxatilis (striped bass-larvae)	static, lab	1.0 (24-96 h)		70°F			Hughes (1973)
(fingerlings) (fry)	static, lab	35.0 (24 h) 10.0 (96 h)		70°F			Hughes (1973)
(fingerlings)	static, lab.	315.0 (24 h) 80.0 (96 h)	8.2	21°C	64	35	Wellborn (1969)

Species	Toxicity test	LC 50[2/] (mg/L)	pH	Temperature	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)	Reference
Oncorhynchus kisutch (coho salmon-yearlings)	static, lab.	30 (96 h)	7.4-7.6	10°C	79-82	100-101	Lorz et al. (this study)
O. tshawytscha (chinook salmon)	static, lab.	29.5 (24 h) 28.5 (48 h)	7.4-7.7	20°C	41-71		Bond et al. (1960)
O. tshawytscha	static, lab.	29.0 (48 h)					Muirhead-Thompson (1971)
Pimephales promelas (fathead minnow)	static, lab.	10.0 NTE (96 h)[5/]		75°F			Lawrence et al. (1965)
P. promelas	static, lab static, lab	14 (96 h) 130 (96 h)	7.4 8.2	25°C 25°C	22 299	22 379	Surber and Pickering (1962)
Rasbora hetermorpha (harlequin fish)	flowing, lab.	73-93 (48 h)		20°C		20	Alabaster (1969)
Salmo gairdneri (rainbow trout)	static, lab.	5.0 LD_{10} (96 h)[5/]		65°F			Lawrence et al. (1965)
S. gairdneri	flowing, lab	70 (48 h)		20°C		250	Alabaster (1969)
S. gairdneri	static, lab	10 NTE[3/]		55°F			U.S. Fish & Wild. Service (1963)
S. gairdneri	static, field concrete pond	11.2 (96 h)	8.2	48-89°F	233	280	Gilderhus (1967)
S. gairdneri	static, lab.	20.0 (48 h)		13°C			Cope (1966)
S. gairdneri	flowing, lab.	10.0 NTE[3/]	8.0			89.5	Folmar (1976)
S. trutta (brown trout-fingerlings)	static, lab. static, lab.	32.6 (24 h) 20.4 (96 h)	7.5	18.3°C	100		Simonin & Skea (1977)
S. trutta	static, lab.	300 (48 h)[6/] 570 (48 h)[6/]	7.6-8.0	10 °C		210-290	Woodiwiss & Fretwell (1974)
Stizostedion vitreum (walleye)	static, lab.	2.1 (96 h)	8.2	48-89°F	233	280	Gilderhus (1967)

[1/] *Modified from Folmar 1977.*
[2/] *Concentration of diquat causing 50% mortality of exposed fish in time given.*
[3/] *NTE = No toxic effect.*
[4/] *Test carried out in seawater.*
[5/] *Approximate threshold toxicity LD_{10} for 96 h contact period.*
[6/] *Concentration of Aquacide and Reglone given as 48 H LC50 values, respectively.*

chemical by mud particles . Woodiwiss and Fretwell (1974) estimated a 48-h LC50 of 300 mg/L diquat for brown trout in relatively hard water; however, this is more than ten-fold higher than that given by Simonin and Skea (1977) of 20.5 mg/L in water about one-half as hard. The majority of the researchers (Table 21) noted that salmonids had a 96-h LC50 of 11-32 mg/L diquat in moderately hard water. Bond et al. (1960) reported a 48-h LC50 of 28.5 mg/L diquat for chinook salmon whereas in our study, we estimated the 96-h LC50 to be 30 mg/L for coho salmon. We did not test concentrations >20 mg/L; and thus the shape of the toxicity curve may be considerably steeper and would give a lower LC50 value. Surber and Pickering (1962) indicated the importance of hardness, noting that the 96-h LC50 of diquat in hard water was 2 to 10 times the level observed in soft water tests with fathead minnows and bluegill sunfish.

Diquat has been shown to be toxic to fish fry. Bluegill, lake chub sucker, and smallmouth bass fry survived for 3 days or less in 2.5 mg/L diquat under static conditions (Hiltibran 1967). Folmar (1976) reported that rainbow trout fry displayed no avoidance of 0.1, 1.0 or 10 mg/L diquat.

Berry et al. (1975) found that treatment of Chickahominy Reservoir, Virginia, with diquat resulted in diquat accumulation in the hydrosol. Similar to other researchers, they found diquat was strongly adsorbed to soil particles and desorption did not appear to occur.

Diquat has been found to be absorbed by fishes, but it does not appear to be cumulative as the herbicide is believed to be metabolized or voided soon after it disappeared from the water (Beasley et al. 1965, Calderbank 1972 and Newman 1970). Beasley et al. (1965) injected two goldfish intraperitoneally with C^{14} labeled diquat and allowed them to swim freely in freshwater for 24 and 48 h. Two additional fish were exposed to 4 mg/L diquat (C^{14} labeled) for 24 and 72 h. The measured radioactivity suggested that diquat moved primarily in the plasma and accumulated predominantly in the gastro-intestinal tract. Placement of fish in freshwater decreased the labeled herbicide in various organs and tissues, but not in the gastro-intestinal tract, and increased the radioactivity of the freshwater.

Experimental Results

Static exposure--

In our study the 96-h LC50 of diquat to yearling coho salmon was estimated to be 30 mg/L and the 144-h LC50 was 19 mg/L. When survivors of static diquat exposure tests were placed in seawater, deaths occurred in a dose dependent manner in all groups of yearling coho exposed to concentrations greater than 1.0 mg/L (Fig. 11, Table 22). Although our sample size was small, there was no apparent effect of diquat on the (Na,K)-stimulated ATPase activity of the gills.

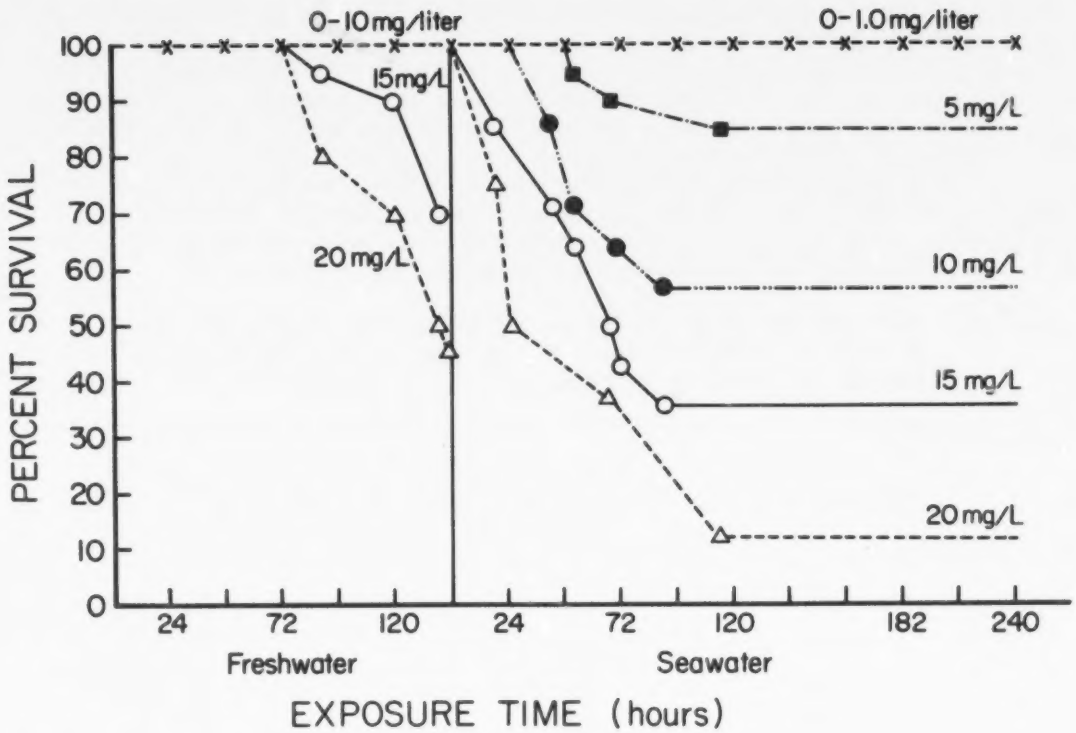

Figure 11. Percent survival of yearling coho salmon during exposure to diquat in freshwater and subsequent survival upon transfer to seawater.

TABLE 22. SURVIVAL AND GILL ATPASE OF YEARLING COHO SALMON EXPOSED TO DIQUAT IN FRESHWATER AND THE SUBSEQUENT SURVIVAL FOLLOWING TRANSFER TO SEAWATER (APR. 19-MAY 5, 1977).

Concentration (mg/L)		Percent survival[a]	Gill	Percent survival
nominal	measured	(144-h exposure FW)	ATPase[b]	(240-h SW)
Control	-	100	5.0 (4)	100
0.25	0.23	100	NT	100
0.50	0.47-0.49	100	NT	100
1.0	1.05-1.06	100	4.9 (5)	100
5.0	4.94	100	NT	85
10.0	9.45	100	4.5 (6)	57
15.0	14.7	70	NT	36
20.0	19.4-19.7	42	NT	13

[a] *Twenty fish exposed per concentration.*
[b] *(Na,K)-stimulated ATPase activity of gill; mean with sample size in parentheses.*

Flow-through exposure--

Additional tests were conducted with diquat because it is used as a prophylactic treatment in fish culture and for control of aquatic vegetation, and affects seawater survival of coho smolts. Following tank acclimatization, 150 yearling coho salmon per tank were exposed to diquat starting May 13, 1977. After 144 h and 312 h of diquat exposure, groups of yearling coho were transferred to seawater. Only one fish died (from the highest toxicant concentration 3 mg/L for 312 h) during the seawater challenge (Table 23). The toxicant concentrations of our composite water samples were close to the desired concentrations. No apparent effect of diquat on the (Na,K)-stimulated ATPase activity was noted (Table 23).

TABLE 23. SURVIVAL AND GILL ATPASE OF YEARLING COHO SALMON EXPOSED TO DIQUAT IN FRESHWATER AND THE SUBSEQUENT SURVIVAL FOLLOWING TRANSFER TO SEAWATER.

Concentration (mg/L) nominal	measured	Percent survival[a/]	Gill ATPase[b/]	Percent survival (268-h SW)
A. 144-h exposure				
Control	0	100		100 (21)[c/]
0.5	0.58	100		100 (19)
1.0	1.07	100		100 (20)
2.0	2.04	100		100 (20)
3.0	3.06	100		100 (20)
B. 312-h exposure				(240-h SW)
Control	0	100	4.93 (20)	100 (20)
0.5	0.44-0.58	100		100 (29)
1.0	0.92-1.04	100	5.83 (20)	100 (20)
2.0	1.91-2.04	100		100 (31)
3.0	2.86-3.06	100	4.28 (20)	95 (19)

[a/] *300 fish exposed per concentration.*
[b/] *(Na,K)-stimulated ATPase activity of gill; mean with sample size in parentheses.*
[c/] *Number of fish tested in parentheses.*

Exposure to diquat for 285 h showed no apparent effect on the condition factor of yearling coho salmon (Table A-7). Generally, the fish appeared to feed well in all tanks, although those fish exposed to 3 mg/L exhibited extensive exophthalmia and opaqueness of the eyes.

A release of 10 groups of yearling coho salmon exposed to diquat for 285 h and 5 groups exposed for 96 h was made on May 26, 1977. An inhibitory effect on migration occurred in all concentrations of diquat tested, in both the acute and chronic exposures (Fig. 12). The coho salmon exposed

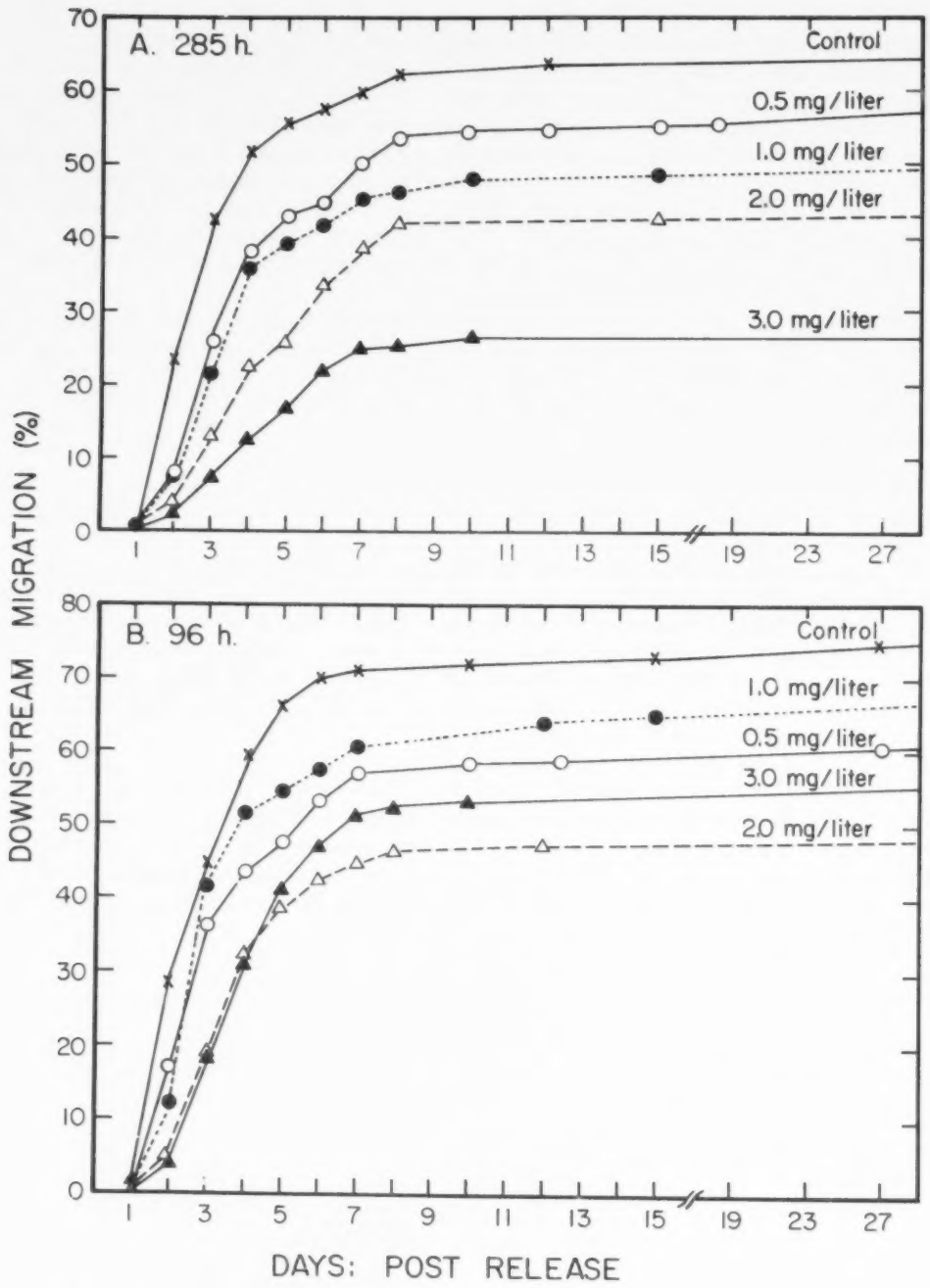

Figure 12. Percent downstream migration of yearling coho salmon following exposure to diquat. A. represents 178-183 coho salmon released per group following 285 h of diquat exposure. B. represents 98-105 coho salmon released per group following 96 h of diquat exposure.

for 285 h showed a dose-dependent inhibition of their migration. Less than 30% of the fish exposed to 3 mg/L diquat migrated the 6.4 km downstream compared to 63% of the controls. The 1, 2, and 3 mg/L groups all showed significantly less migration than the controls ($P = 0.05$). In the 96-h exposure groups the percent migration varied from 48% (2 mg/L) to 72% (controls), but the group responses were not dose-dependent. The 0.5, 2 and 3 mg/L (96-h exposure) groups all exhibited significantly less migration than the controls. The controls showed the best migratory response following both acute and chronic diquat exposure (Table A-8).

Histological examination of diquat-exposed fish showed degenerative necrosis of the liver, kidneys, and gill lamellae of chronically exposed coho salmon (Appendix II). In acute exposures, however gill tissues exhibited the most degeneration although the liver did show limited necrosis (Appendix II).

No bioconcentration of diquat was found in muscle, kidney, or liver tissue, although difficulty was encountered in the extraction procedure. The literature generally indicates that diquat may enter a fish's body but is not accumulated since the herbicide is metabolized or excreted soon after it disappears from the water (Beasley et al. 1965, Calderbank 1972, Newman 1970). However, Gilderhus (1967) found diquat residues in bluegills 6 weeks after treatment with 1.0 ppm. Calderbank 1972, Hiltibran et al. (1972) and Berry et al. (1975) showed that most, if not all, diquat residues in fish were located in skin, gills, and viscera and not in edible flesh.

Considering that diquat is used for both aquatic weed control and in fish culture for treatment of bacterial gill disease, further investigation appears warranted. Information concerning the effect of reduced exposure time or a period of recovery following exposure would permit a better evaluation of diquat's possible adverse effects where migrating salmonids are concerned.

SECTION VI

GENERAL DISCUSSION

We attempted to determine the 96 h-LC50 value of 12 water soluble herbicides to yearling coho salmon. The toxicity of the compounds tested varied from <0.1 to >200 mg/L. Acrolein and dinoseb were the most toxic, with 96-h LC50 values of 68 and 100 µg/L, respectively; ammonium ethyl carbamylphosphonate (Krenite) and 2,4-D (dimethyl amine) were apparently non-toxic at concentrations of 200 mg/L. Several researchers (Hughes and Davis 1963, Meehan et al. 1974 and Woodward 1976) have discussed the importance of alkalinity, hardness, pH, temperature, and especially chemical formulation of the herbicide, as these parameters related to observed toxicity to fishes.

Most fish species, but particularly salmonids, would succumb to concentrations of acrolein or dinoseb if exposed at the approved application rates (Oregon Weed Control Handbook 1977). When coho salmon yearlings were removed from either herbicide and placed in seawater, few additional deaths occurred. The (Na, K)-stimulated ATPase activity of the gills appeared unaffected by either herbicide.

The toxicity of some compounds may be cumulative over periods of chronic exposure. Sprague (1969) and Eaton (1970) emphasized the importance of maintaining experimental exposures long enough to adequately define lethal threshold concentrations. The persistence of dinoseb increases the possibility of continuous aquatic habitat contamination. In our dinoseb flow-through exposures, concentrations of 40 and 60 µg/L resulted in almost total mortality during the 11 days of exposure; whereas, in an earlier static experiment, 75 µg/L for 6 days caused no mortality. Woodward (1976) did not observe cumulative mortalities in cutthroat trout or lake trout exposed to dinoseb for 8 days. Similarly, in our study concentrations of 10 and 20 µg/L for 15 days did not cause any deaths.

Acute exposure of yearling coho salmon to Amitrole-T, diquat and paraquat produced moderate mortality (30-75 mg/L); and the subsequent challenge with seawater elicited a dose-dependent response, even at concentrations that were sublethal in freshwater. No statistically significant effect of the herbicides on (Na,K)-stimulated ATPase activity of the gill was observed. Coho salmon acutely and chronically exposed to diquat exhibited migratory inhibition upon release into a small coastal stream.

Atrazine, Krenite, 2,4-D, and 2,4,5-T exhibited minimal or no effects on smolting of yearling coho salmon, i.e. little or no mortality in the seawater challenge test, and no effect on (Na,K)-stimulated ATPase activity.

Only atrazine caused deaths of coho salmon during the freshwater exposure (25% mortality at 15 mg/L).

Dicamba (Banvel D), Tordon 22K (picloram) and Tordon 101 (picloram + 2,4-D) all produced unexplainable deaths in seawater of yearling coho salmon previously exposed to only the lowest concentration of the herbicide. No effect on the (Na,K)-stimulated ATPase activity of the gills was noted although fish from concentrations causing mortality in seawater were not always checked.

Acute (96-h) exposure to Tordon 101 had no apparent effect on migration of yearling coho salmon, whereas the two highest concentrations did elicit a reduced migratory response of chronically exposed fish (15 days at 1.2 and 1.8 mg/L) as compared to control or low Tordon 101 exposure groups. The concentrations of Tordon 101 that produced the inhibitory effect on migration, however, are considerably greater than one would expect to see utilized in a field operation. As noted earlier, picloram is extremely persistent and could be present for up to a year following application.

Generally, except for the direct toxic effect of acrolein and dinoseb and effects of sublethal concentrations of diquat on migration and seawater survival, the herbicide formulations tested did not appear to affect smolting of yearling coho salmon *per se* as measured by seawater tolerance, (Na,K)-stimulated ATPase activity or migratory tendency. In our study, however, we tested only one life stage of the salmonids' life history; whereas genetics, younger life stages, or food resources may be considerably more affected as noted by Macek et al. (1976b) in a study with atrazine.

The insecticides DDT and sumithion (fenitrothion) have been shown to modify salmonid physiology, behavior, and learning ability following exposure to sublethal concentrations (Anderson 1971, Anderson and Peterson 1969, Elson et al. 1972, Hatfield and Anderson 1972, Hatfield and Johansen 1972, Hatfield and Riche 1970, Keenleyside 1967, Saunders 1969, Symons 1973, 1977, Warner et al. 1966 and Wildish et al. 1971). Whether the herbicides tested would elicit responses similar to these insecticides is unknown. It does suggest, however, that appropriate precautions be taken during the application of any herbicide to watersheds containing fish.

SECTION VII

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APPENDIX I. METHODOLOGY FOR ANALYSIS OF SPECIFIC HERBICIDES.

AMITROLE-T

1. Summary of method: The method is an adaptation of Southerland (1964). Aminotriazole is removed from water by adsorption on an ion exchange resin. The eluate from the resin is decolorized with activated charcoal. N-(1-Naphthyl) ethylenediamine dihydrochloride is used to form a chromophoric moiety which is measured in a colorimeter at 460 nm.

2. Reagents: Dowex 50W-X8

3-amino-s-triazole

Sulfuric Acid, reagent grade (concentrated, 75% and 0.75%)

Sodium nitrite, reagent grade (0.5% aqueous solution, prepare fresh daily)

Sulfamic acid, reagent grade (5.0% aqueous solution, prepare fresh daily)

N-(1-Naphthyl) ethylenediamine dihydrochloride, reagent grade (1.0% aqueous solution, prepare fresh daily)

Charcoal, activiated ("Darco" G-60)

Ammonium hydroxide, reagent grade, 2 Normal solution

3. Apparatus: Widemouth plastic bottles, 500 mL (sample containers)

Magnetic stirrers and stir bars
Chromatographic columns
Hot plates
Colorimeter
Timer
Suction flask (500 mL)
Sintered glass funnels (medium porosity)
Beakers (600, 400 & 100 mL)
Erlenmeyer flasks (50 mL)
Volumetric flasks (25 mL)
Pipet
Glass beads (3 mm)

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4. Procedure:

a. Resin adsorption and desorption of aminotriazole: Measure 200 mL of water sample into a 400 mL beaker. Add 10 g Dowex 50W-X8, place on stirrer and stir for 30 min. Set up a chromatographic column with 1 g of resin in the bottom. Rinse the sample and resin from the beaker into the column with distilled water, allow the water to drain through the column and discard the water. Elute the aminotriazole from the resin with 200 mL of 2N ammonium hydroxide into a 600 mL beaker.

b. Acid digestion and cleanup: Add 2 or 3 glass beads and boil on a hot plate until volume is 25-50 mL, cool and add 3 mL of 75% sulfuric acid solution. Boil for 10 min, maintaining volume with water rinsing. Carefully add 1 g charcoal, boil for another 10 min, maintaining volume at 20 to 25 mL with water rinse.

Filter hot solution through sintered glass funnel with suction and rinse with 4x25 mL portions of hot 0.75% sulfuric acid solution followed by 100 mL of hot water.

Boil filtrate down to approximately 50 mL, transfer to a 100 mL beaker and continue boiling down until volume is between 15 and 20 mL. Transfer to a 25 mL volumetric flask with water, rinse beaker and bring to volume.

c. Color development: Transfer a 5 mL aliquot of sample into a 50 mL erlenmeyer, add 3 mL concentrated sulfuric acid and cool in an ice bath. Add 10 drops of 0.5% sodium nitrite, and swirl; after 10 min, add 10 drops of 5.0% sulfamic acid, and swirl. After 10 min add 10 drops 1.0% N-(1-Naphthyl) ethylenediamine dihydrochloride and swirl. Read against a reagent blank at 460 nm within 30 min. Using a standard curve for aminotriazole, determine the amount of 3-amino-s-triazole in the 5 mL aliquot.

ATRAZINE

1. Summary of method: This method is based on Zweig and Sherma (1972a). Atrazine is extracted from water with methylene chloride. Analysis is carried out using a chloride specific microcoulometric gas chromatographic system.

2. Reagents: Methylene chloride, reagent grade

Methanol, reagent grade

3. Apparatus:
Glass jars with teflon-lined caps
Separatory funnels (500 mL)
Graduated cylinders (500 & 100 mL)
Beakers (250 mL)
Volumetric flasks
Gas chromatograph, MicroTek 2000MF, or equivalent, with Dohrmann model C200B microcoulometric detector with halogen titration cell

4. Procedure:

a. Extraction: Measure 400 mL of water sample into 500 mL separatory funnel fitted with teflon stopcock. Shake sample with three successive 75 mL portions of methylene chloride combining the three portions in a 250 mL beaker. Evaporate the methylene chloride to near dryness using a stream of nitrogen. Add 20 mL methanol and evaporate to near dryness again to ensure all methylene chloride is removed.

Transfer to volumetric flask with methanol.

b. Gas Chromatography: Gas chromatograph parameters:

Coulometer settings:	250 Ω, 250 mV bias, low gain mode.
Column:	4 mm I.D. x 1.2 m glass packed with 5% OV-1 on 80/100 Gas Chrom Q.

Temperatures

Column	175°C
Inlet	205°C
Transfer line	225°C
Combustion furnace:	800°C
Gas flows:	Carrier gas 60 mL nitrogen/min, oxygen 50 mL/min. Sweep gas 20 mL nitrogen/min.
Recorder:	0.1 mV/in (0.04 mV/cm), 1/2 in/min (1.27 cm/min) chart speed. Equipped with a disc integrator.
Retention time of atrazine:	2.8 min.

The amount of atrazine in the samples is determined by injecting an aliquot of the methanol solution into the gas chromatograph. Compare peak chart areas with areas for known amounts of atrazine.

DICAMBA, PICLORAM, 2,4-D, and 2,4,5-T

Residues in water

1. Summary of method: Samples are collected and stabilized with sodium hydroxide. The samples are adjusted to a pH <2, extracted with ether and alkylated to form the methyl ester. Detection and quantification is accomplished using microcoulometric gas chromatography (MCGC) in the halogen mode.

2. Reagents:

Diethyl ether (distilled over sodium)
Sulfuric acid (concentrated, reagent)
Sodium hydroxide (reagent)
Diazomethane
BF_3-methanol

3. Apparatus:

Widemouth plastic bottles (500 mL)
Separatory funnels fitted with Teflon stopcocks
Beakers
Volumetric flasks
Graduated cylinder
Gas chromatograph - MicroTek 2000 MF, or equivalent, with Dohrman model C200B microcoulometric detector with halogen titration cell.

4. Procedure:

a. Sample collection: water samples are collected in 500 mL widemouth plastic bottles which contain 3 mL of 50% sodium hydroxide. The samples are mixed to ensure complete hydrolysis of any of the herbicide ester which might be present. The samples are transported to the laboratory and stored at 2°C.

b. Sample extraction: Transfer 200 mL of water sample to a 500 mL separatory funnel. Adjust to pH < 2 with concentrated sulfuric acid. Extract with three successive aliquots of ether (100, 80, 60 mL). The ether extracts are collected in a 250 mL beaker, and evaporated to less than 20 mL. The ether layer is carefully decanted away from any water and into a 30 mL beaker. The residual extract is rinsed with three small aliquots of ether adding the rinsings to the beaker. The extract is evaporated to 2 mL with a stream of dry nitrogen. This concentrated extract is transferred with small aliquots of ether into a 10 mL volumetric flask, carefully decanting away from any water and evaporated to dryness on a flash evaporator.

c. Derivation: The methyl esters of dicamba, and picloram are formed using an ethereal diazomethane reagent. The methyl esters of 2,4-D and 2,4,5-T may be formed using the diazomethane reagent (Hopps, 1970) or a BF_3-methanol reagent (Burchfield and Johnson, 1965).

d. Gas chromatography:

Gas chromatography parameters:

Coulometer settings:	300 Ω, 250 mV bias, low gain mode.
Column:	4 mm I.D. x 1.2 m glass packed with 5% OV-1 on 80/100 Gas Chrom Q.

Temperatures

Column	200°C for 2,4-D, 2,4,5-T, and picloram 185°C for dicamba.
Inlet	205°C
Transfer line:	225°C
Combustion furnace:	825°C
Gas flow:	Carrier gas 60 mL nitrogen/min, Oxygen 50 mL/min. Sweep gas 21 mL nitrogen/min.
Recorder:	0.1 mV/in (0.04mV/cm) 1/2 in/min (1.27 cm/min), chart speed. Equipped with a disc integrator.

Retention time of methyl esters:	dicamba	2.0 min (185°C)
	2,4-D	2.0 min (200°C)
	2,4,5-T	3.0 min (200°C)
	picloram	5.5 min (200°C)

The amount of herbicide in the samples is determined by injecting an aliquot of sample solution in the gas chromatograph and comparing peak chart areas with areas for known amounts of the herbicide injected.

PICLORAM, 2,4-D, AND 2,4,5-T

Residues in Fish

1. Summary of method: Whole body fish samples are digested with potassium hydroxide and centrifuged. The supernate is adjusted to pH <2, extracted with ether and alkylated to form the methyl ester. Detection and quantification is accomplished using microcoulometric gas chromatography (MCGC) in the halogen mode.

2. Reagents:
   Diethyl ether (distilled over sodium)
   Sulfuric acid (concentrated, reagent)
   Potassium hydroxide (reagent)
   Diazomethane
   95% ethanol

3. Apparatus:
   Separatory funnels fitted with teflon stopcocks
   Round-bottom boiling flask (250 mL)
   Condenser (West, 24/40 ℨ)
   Volumetric flask, 10 mL

Graduated cylinder, 100 mL
Heating mantle, 250 mL
Centrifuge (International Model U or equivalent)
Centrifuge bottles, 250 mL
Beakers
Flash evaporator (CaLab Model 5101 or equivalent)
Tissue grinder (Tek Mar Model SDT or equivalent)
Gas chromatograph - MicroTek 2000 MF, or equivalent, with Dohrmann model C200B microcoulometric detector with halogen titration cell.

4. Procedure:

a. Sample extraction: Weigh sample, cut in small pieces and place in 250 mL round-bottom boiling flask. Add 50 mL of 95% ethanol, grind with tissue grinder. Add 100 mL 50% potassium hydroxide in water solution, place in a heating mantle and attach a reflux condenser. Reflux for one hour. Cool, transfer contents to centrifuge bottle and centrifuge at 2000 rpm for 20 min. Decant into separatory funnel, acidify to <pH 2.0 with concentrated sulfuric acid, and extract with three aliquots (100, 75, 60 mL) of ether. Evaporate to 2 mL with a stream of dry nitrogen and transfer with small aliquots of ether to a 10 mL volumetric flask. Evaporate to dryness using a flash evaporator. The derivation, gas chromatography and quantification are identical with the method for water analysis.

DINOSEB

Residues in water

1. Summary of method: The method is based on Zweig and Sherma (1972b). Samples are collected and stabilized with sodium hydroxide. The samples are adjusted to pH < 2, extracted with ether and alkylated to form the methyl ester. Detection and quantification is accomplished using electron-capture gas chromatography (ECGC).

2. Reagents:

Diethyl ether (distilled over sodium)
Sulfuric acid (concentrated, reagent)
Sodium hydroxide (reagent)
Diazomethane

3. Apparatus:

Glass jars with teflon lined caps
Separatory funnels fitted with teflon stopcocks
Beakers
Volumetric flasks
Graduated cylinders
Gas chromatograph - Varian Model 1200 or equivalent with scandium E.C. detector.
Flash evaporator (CaLab Model 5101 or equivalent)

4. Procedure:

a. Sample collection: Water samples are collected in 500 mL, wide-mouth, glass bottles which contain 3 mL of 50% sodium hydroxide. The samples are mixed to ensure complete hydrolysis of any DNBP which may be present. The samples are transported to the laboratory and stored at 2°C.

b. Sample extraction: Transfer 200 mL of water sample to a 500 mL separatory funnel. Adjust to pH < 2 with concentrated sulfuric acid. The acidified sample is extracted with three successive aliquots of ether (100, 80, 60 mL). The ether extracts are collected in a 250 mL beaker, evaporated to less than 20 mL. The ether layer is carefully decanted away from any water into a 30 mL beaker. The residual extract is rinsed with three small aliquots of ether adding the rinsings to the beaker. The extract is evaporated to 2 mL with a stream of dry nitrogen. This concentrated extract is transferred with small aliquots of ether into a 10 mL volumetric flask, decanting away any water and evaporated to dryness on a flash evaporator.

c. Derivation: The methyl ester of DNBP is formed using an ethereal diazomethane reagent (Hopps 1970).

d. Gas chromatography: Gas chromatograph parameters:

Column:	4 mm I.D. x 1.2 m glass, packed with equal amounts of 6% OV 210 and 4% SE 30 on 80-100 Gas Chrom Q.
Temperatures	
Column	180°C
Inlet	200°C
Gas flow:	Carrier gas 80 mL nitrogen/min.
Recorder:	0.1 mV/in (0.04mV/cm), 1/2 in/min (1.27cm/min) chart speed.
Retention time of DNBP methyl ester:	3.5 min.

The amount of DNBP in the samples is determined by injecting an aliquot of sample solution in the gas chromatograph and comparing peak heights with peak heights for known amounts of injected DNBP.

Residues in Fish

1. Summary of Method: The method is based on Lane (1967) and Zweig and Sherma (1972b). Whole body fish samples are digested with potassium hydroxide and centrifuged. The supernate is adjusted to pH <2, extracted with benezene and alkylated to form the methyl ester. The ester is cleaned up with an alumina column. Detection and quantification is accomplished using electron-capture gas chromatography.

2. Reagents:
Diethyl ether (distilled over sodium)
Benzene (Burdick & Johnson, Distilled in Glass TM)
Potassium hydroxide (reagent)
Sulfuric acid (concentrated, reagent)
Alumina, activated (Fisher A-540)
Diazomethane
n-Hexane (distilled over sodium)
Acetone (Burdick & Jackson, Distilled in Glass TM)

3. Apparatus:
Separatory funnels fitted with teflon stopcocks
Beakers
Volumetric flasks
Graduated cylinders
Pipet, Pasteur, disposable
Heating mantle, 250 mL
Round-bottom boiling flask (250 mL)
Condenser (West, 24/40 ȝ)
Centrifuge (International Model U or equivalent)
Centrifuge bottles, 250 mL
Tissue grinder (Tek Mar Model SDT or equivalent)
Gas chromatograph (Varian Model 1200 or equivalent with scandium E.C. detector)
Flash evaporator (CaLab Model 5101 or equivalent)

4. Procedure:

a. Sample extraction: Weigh sample, cut in small pieces and place in 250 mL round-bottom boiling flask. Add 50 mL of 95% ethanol, grind with tissue grinder. Add 100 mL 50% potassium hydroxide in water solution, place in a heating mantle and attach a reflux condenser. Reflux for 1 hour. Cool, transfer contents to centrifuge bottle and centrifuge at 2000 rpm for 20 min. Decant into separatory funnel, acidify with concentrated sulfuric acid, and extract with three 75 mL aliquots of benzene. Evaporate the extract to dryness with the aid of a flash evaporator at 40°C.

b. Derivation and cleanup: Add the ethanol solution of diazomethane (Hopps 1970) drop by drop until the yellow color persists. Swirl the flask gently, allow to stand 10 min, and evaporate just to dryness. Prepare the alumina column by tamping a plug of glass wool into a disposable Pasteur pipet and pour in a 1-in layer of activated alumina. Transfer the methylated residue to the column with two 3 mL portions of hexane. Discard the hexane eluate. Elute the DNBP methyl ester with 4 mL ethyl ether. Collect the ether in a 5 mL volumetric flask.

c. Gas chromatography and quantification are identical with the method for water analysis.

DIQUAT AND PARAQUAT

This method is taken from Pack (1967). The control water samples were clear and contained no absorbing species at 310 nm or 256 nm, therefore, we were

able to determine concentrations of diquat by direct readings of the water at 310 nm and for paraquat at 256 nm. Concentration was determined from a standard curve.

APPENDIX II. EFFECT OF VARIOUS HERBICIDES ON HISTOLOGY OF YEARLING COHO SALMON. BY DR. J. D. HENDRICKS, OREGON STATE UNIVERSITY.

ACROLEIN

Control, 144 h (3 fish) - All tissues were normal.

50 µg/L acrolein, 144 h (3 fish) - The livers had occasional exfoliated, necrotic cells, but were otherwise normal. One of the kidneys was normal, one had extensive vacuolation of the collecting duct cells, while the other had protein precipitate and cellular debris present in Bowman's capsule and various tubular regions. Both hypertrophy and hyperplasia were seen in the gill epithelium of fish examined.

100 µg/L acrolein, 44 h (3 fish) - Most of the liver cells were delineated and separate from adjacent ones, some were necrotic. All the kidneys had considerable debris and precipitate in tubule lumens, and one kidney had extensive necrosis of both segments of the proximal tubule. All kidneys were engorged with blood. The gill epithelium from all fish was totally destroyed, necrotic and sloughed. Two hearts were normal. A section of esophagus exhibited massive necrosis of all 4 tunics.

AMITROLE-T

Control, 144 h (3 fish) - There was extensive peripheral, coagulative necrosis due to bile spillage and several foci of peribiliary necrosis within the substance of the liver. The remaining liver cells were normal, so I assume both of these lesions were the result of poor post-mortem handling of the livers. Kidney and gill tissues were normal.

100 mg/L Amitrole-T, 144 h (1 fish) - All liver cells showed either hydropic degeneration or coagulative necrosis. The necrosis was diffuse; approximately 25% of the parenchymal cells were affected. There was extensive coagulative necrosis of all regions of the nephrons as well as most of the hematopoietic tissue of the kidney. The lamellar epithelium of the gills was hydropic and separated from the underlying pillar cells on many of the lamellae. On the basis of the effects on these 3 tissues, this treatment was severe and the changes would be incompatible with survival.

200 mg/L Amitrole-T,60 h (3 fish) - Changes in the liver were of the same type as in the 100 ppm exposure but not as extensive. One of the fish had considerable diffuse coagulative necrosis of liver cells, but the others showed predominantly hydropic degeneration. Kidneys exhibited similar necrotic changes in the tubules and hematopoietic tissue, but not quite as extensive. Epithelial cells of the gills were hypertrophied, disrupting normal lamellar architecture. Although not as severe as the previous treatment, these tissue changes would probably not permit longtime survival.

ATRAZINE (AATREX)

15 mg/L AAtrex, 140 h (3 fish)- The livers and kidneys of these fish were normal. There was hypertrophy of the gill epithelium in two of the fish, while the third had several large aneurisms at the base of the filaments.

DICAMBA (BANVEL D)

100 mg/L dicamba, 144 h (5 fish) - All livers exhibited foci of peripheral and/or peribiliary bile necrosis but were otherwise normal. Kidneys and gills were also normal in these fish. The experimental treatment had little if any effect on the tissues examined.

PARAQUAT

100 mg/L paraquat, 120 h (4 fish) - All the livers exhibited a low grade hydropic degeneration, particularly in centrolobular regions and occasionally in foci of peribiliary necrosis. One kidney was normal, the other 3 had necrotic cells in the first and second proximal tubules. Two of the fish had gills from which nearly all the epithelium had been sloughed; where it remained, the epithelium was degenerate or necrotic. The other two fish had less severe gill lesions.

ESTERON [BRUSH KILLER (2,4-D + 2,4,5-T PGBE ESTER)]

Control, 96 h (5 fry)[1/]- Livers and kidneys were normal on all these fish, but the gills were similar to the experimentals with regard to chloride cell hyptertrophy. This response may be the result of something other than the Esteron treatment. Its significance is not clear.

1200 µg/L Esteron, 28 h (5 fry)[1/]- Some peribiliary necrosis was present in all livers and some diffuse cellular degeneration was observed in two of them. Kidneys were normal in all fish, but gills had lesions similar to the 800 µg/L treatment, i.e. hypertrophied chloride cells and lamellae engorged with blood.

1200 µg/L Esteron, 48 h (4 fry)[1/]- Livers were normal except some slight degenerative changes in one. Kidneys were again normal and gills had similar lesions to the 2 previous treatments, i.e. hypertrophied chloride cells and blood engorged lamellae.

1200 µg/L Esteron, 96 h + 24 h rest, (5 fry)[1/]- All tissues were normal except for foci of peribiliary necrosis in 3 livers.

Control (yearling coho salmon), 96 h (3 fish) - All tissues were normal except for some peripheral bile necrosis on the livers.

[1/] *Steelhead fry - Big Creek Salmon Hatchery.*

800 µg/L Esteron 96 h (3 fish) - Except for peripheral bile duct necrosis livers were normal in all samples. Kidneys were normal with the exception of large clear vacuoles in the collecting duct cells from two fish. The gills had several abnormalities; 2 of them had curved gill filaments, the basal, interlamellar epithelium had frequent hypertrophied chloride (acidophillic) cells, some of which were exfoliated, and most of the lamellae were engorged with blood, resulting in large aneurisms on some lamellae.

TORDON 22K (PICLORAM)

Control, 144 h (3 fish) - Livers had some foci of both peripheral and peribiliary bile necrosis. The hematopoietic tissue of the kidneys appeared depleted and some of the gill filaments were crooked. Lamellar epithelium appeared normal.

5 mg/L picloram, 144 h (4 fish) - Extensive degenerative changes were present in the liver, some hydropic in nature and some similar to cyclopropene fatty acid-induced damage. Cells were hypertrophied and contained fiber-like strands in the cytoplasm. Kidneys were normal, while gills had several abnormalities including hypertrophy of epithelial cells, and a wrinkled appearance of many of the epithelial cells.

TORDON 101 (2,4-D + PICLORAM)

Control, 380 h (3 fish) - Livers were normal except that one had peripheral bile necrosis. There were large clear vacuoles in the collecting duct cells of one kidney, others were normal. The gill epithelium was wrinkled as though it had been stretched and then shrunk.

1.8 mg/L Tordon 101, 380 h (4 fish) - All livers had degenerate bile duct epithelium and resulting extensive peribiliary bile necrosis. Since the bile ducts were affected, this may be due to the herbicide. Kidneys and gills were normal.

1.2 mg/L Tordon 101, 380 h (4 fish) - All livers had degenerate bile duct epithelium and peribiliary bile necrosis. All kidneys and gills were normal except that there was considerable separation of epithelial cells from the pillar cells in the lamellae in one fish.

0.6 mg/L Tordon 101, 380 h (4 fish) - Peribiliary bile necrosis was present in all livers as above, kidneys and gills were normal.

0.3 mg/L Tordon 101, 380 h (4 fish) - Peribiliary bile necrosis was present in all livers as above, kidneys and gills were again normal. All levels of Tordon 101 caused peribiliary necrosis, this was not present in the controls so it was assumed that it was due to the treatment.

DINOSEB

Control, 144 h (3 fish) - Some peripheral and peribiliary bile necrosis of the livers was present but otherwise they were normal. Kidneys and gills were normal.

40 µg/L dinoseb, 336 h (3 fish) - There was some hydropic change in one of the livers and occasional scattered necrotic cells. The other two livers were normal. The second segment of the proximal tubule had occasional cells with pyknotic nuclei and first proximal segment cells contained eosinophilic droplets. Gills were normal, tissue changes from this treatment were minor.

60 µg/L dinoseb, 168 h (6 fish) - Livers, kidneys and gills from all fish were normal. There appeared to be no effect on these tissues.

100 µg/L dinoseb, 114 h (4 fish) - All the livers had peripheral bile necrosis and extensive diffuse necrosis of the remaining parenchymal cells. All regions of the kidney tubules as well as most of the hematopoietic tissue were necrotic. The gill epithelium was totally necrotic and sloughed from the gill lamellae. This treatment was severe and extremely toxic to the tissues examined.

DIQUAT

Control, 360 h (5 fish) - Livers, kidneys and gills of these fish were normal.

10 mg/L diquat, 144 h (3 fish) - Livers showed hydropic degenerative changes in centrolobular regions and some necrosis in one liver. Kidneys of all three fish were normal but gills showed both limited hypertrophy and hyperplasia of epithelial cells.

20 mg/L diquat, 144 h (1 fish) - This fish had extensive hydropic degeneration and necrosis of the liver. The kidney was normal but the gills had both hypertrophy and hyperplasia of epithelial cells.

3 mg/L diquat 360 h (5 fish) - Several foci of degenerate and occasionally necrotic cells were present in the liver. The kidneys also had numerous degenerate and some necrotic tubule cells, particularly in the collecting ducts. Both hypertrophy and hyperplasia of lamellar and interlamellar epithelium were present in the gills.

APPENDIX III. METRIC AND ENGLISH EQUIVALENTS

English-to-Metric		Metric-to-English	
1 inch	= 2.5400 cm	1 centimeter	= 0.3937 in
1 inch^3	= 16.3871 cm^3	1 centimeter^3	= 0.0610 in^3
1 foot	= 0.3048 m	1 meter	= 3.2808 ft
1 foot^2	= 0.0929 m^2	1 meter^2	= 10.7639 ft^2
1 chain	= 20.1207 meter	1 meter	= 0.0497 chain
1 acre	= 0.4047 ha	1 hectare	= 2.4710 ac
1 foot^2/acre	= 0.2296 m^2/ha	1 meter^2/hectare	= 4.3560 ft^2/ac
1 gallon(U.S.liquid)	= 3.7853 L	1 liter	= 0.2642 gallon(U.S.liquid)
1 pound	= 453.5924 gms	1 gram	= 0.0022 pounds
Degrees Fahrenheit	= 9/5 (^{o}C) + 32	Degrees Celsius	= 5/9 (^{o}F - 32)

1 meter = 100 centimeters
1 meter = 1,000 millimeters
1 hectare = 10,000 m^2

TABLE A-1. CONCENTRATIONS OF 2,4-D AND 2,4,5-T IN STEELHEAD TROUT FRY FOLLOWING EXPOSURE TO ESTERON BRUSH KILLER[a]

Exposure concentration (µg/L)		Time	Wet weight of	Residue found (mg/kg)	
nominal	measured	(h)	tissue (g)	2,4-D	2,4,5-T
A. May 31-June 4, 1977					
Control	32.9-34.0	96	15.27	<0.05	<0.06
75	35.2-37.2	96	10.30	0.90	1.92
450	77.4	96	11.48	41.0	44.4
450	80.1	96	12.20	23.1	40.8
800	[b]	48	10.65	8.70	10.6
800	[b]	48	11.07	19.57	26.28
1200	[b]	48	10.75	8.28	7.81
1200	[b]	48	4.06	1.48	6.61
1200	(+ 48 h clean water)[b]	48	4.49	3.39	9.26
800	(mortalities)[b]	24	1.32	64.5	60.4
1200	(mortalities)[b]	24	16.78	16.47	100.5
B. June 12-16, 1977					
Control	6.5-7.2	96	25.11	-	-
800	202-269	96	19.6	5.12	13.94
1000	240-243	96	18.85	7.66	24.37
1200	226-246	96	19.98	11.35	25.13

[a] *Whole body residues.*
[b] *Samples lost during extraction.*

TABLE A-2. SURVIVAL AND GILL ATPASE ACTIVITY OF YEARLING COHO SALMON EXPOSED TO TORDON 101 IN FRESHWATER AND SUBSEQUENT SURVIVAL FOLLOWING TRANSFER TO SEAWATER.

Concentration (mg/L)[a] nominal	measured	Percent survival	Gill ATPase[a/b]	Percent survival[a] (240-h SW)
A. 144 h exposure (Mar. 28, 1977-Apr. 13, 1977)				
Control (220)	0.02	100	3.64 ± 1.46 (19)	100 (20)
0.2 (220)	0.34-0.36	100		100 (22)
0.6 (220)	0.32-0.49	100	3.10 ± 1.00 (19)	100 (22)
1.2 (220)	1.07-1.24	100		100 (22)
1.8 (220)	0.94-1.25	100	3.93 ± 1.55 (20)	100 (22)
B. 380 h exposure (Mar. 28, 1977-Apr. 25, 1977)				
Control (180)	0.005	100	3.00 ± 0.86 (19)	100 (30)
0.3 (180)	0.26-0.39	100	2.54 ± 0.99 (19)	100 (30)
0.6 (180)	0.50-0.52	100	2.30 ± 0.91 (20)	100 (30)
1.2 (180)	1.33-1.40	100	3.06 ± 1.40 (20)	100 (30)
1.8 (180)	1.23-1.39	100	2.43 ± 0.80 (20)	96.7 (30)[c]

[a] *Number of fish used in parenthesis.*

[b] *(Na,K)-stimulated ATPase activity of gill; mean ± SE; number of fish sampled in parenthesis.*

[c] *Exposed for 480 h to seawater as fish appeared to behave differently than other groups. One death occurred after 400 h of exposure.*

TABLE A-3. EFFECT OF TORDON 101 EXPOSURE ON AVERAGE LENGTH, WEIGHT, AND CONDITION FACTOR.

Date of sample	Days of exposure	Tank No.	Nominal concentration (mg/L)	Fork length cm ± SE	Weight g ± SE	Condition factor KFL ± SE
Mar. 9, 1977	0[a]	1	Control	15.5 ± 0.32	42.9 ± 2.42	1.118 ± 0.012
	0	2	Control	15.1 ± 0.17	39.9 ± 1.29	1.163 ± 0.013
	0	7	0.3	14.8 ± 0.29	37.9 ± 2.16	1.134 ± 0.009
	0	8	0.3	15.2 ± 0.26	40.5 ± 1.86	1.145 ± 0.010
	0	3	0.6	14.8 ± 0.28	37.6 ± 2.10	1.125 ± 0.009
	0	4	0.6	15.2 ± 0.22	40.4 ± 1.63	1.130 ± 0.012
	0	5	1.2	15.1 ± 0.25	39.2 ± 1.83	1.118 ± 0.011
	0	6	1.2	15.4 ± 0.20	41.4 ± 1.49	1.111 ± 0.012
	0	9	1.8	14.9 ± 0.23	39.0 ± 1.85	1.156 ± 0.014
	0	10	1.8	14.9 ± 0.17	37.4 ± 1.23	1.125 ± 0.009
Apr. 12, 1977	0[b]	1	Control	15.7 ± 0.21	39.1 ± 1.58	0.998 ± 0.007
	0	2	Control	15.4 ± 0.17	37.9 ± 1.14	1.022 ± 0.008
	15	7	0.3	15.6 ± 0.19	39.9 ± 1.64	1.035 ± 0.011
	15	8	0.3	15.5 ± 0.18	39.2 ± 1.34	1.043 ± 0.009
	15	3	0.6	15.5 ± 0.25	39.6 ± 1.88	1.024 ± 0.008
	15	4	0.6	15.6 ± 0.24	40.1 ± 1.96	1.036 ± 0.011
	15	5	1.2	14.7 ± 0.16	33.5 ± 1.18	1.038 ± 0.018
	15	6	1.2	15.6 ± 0.23	41.7 ± 1.98	1.067 ± 0.021
	15	9	1.8	15.3 ± 0.20	36.9 ± 1.47	1.016 ± 0.011
	15	10	1.8	15.4 ± 0.21	38.5 ± 1.55	1.038 ± 0.009

[a] *Sample size 30*
[b] *Sample size 40*

TABLE A-4. PERCENT MIGRATION (TO JULY 6, 1977) OF YEARLING COHO SALMON RELEASED INTO A SMALL COASTAL STREAM FOLLOWING ACUTE AND CHRONIC EXPOSURE TO TORDON 101 (RELEASED APRIL 13, 1977).

Nominal concentration (mg/L)	Percent migration Days post release				
	1-5	6-10	11-20	21-30	31+
1. Acute exposure - 96 h					
Control	60.9	63.6	63.6	63.6	63.6
0.3	59.8	64.5	65.4	67.3	67.3
0.6	50.4	51.3	52.2	53.9	53.9
1.2	55.0	56.0	56.0	56.9	56.9
1.8	61.5	62.4	62.4	62.4	62.4
2. Chronic exposure - 360 h					
Control	46.0	47.9	48.3	48.8	48.8
0.3	48.4	52.6	54.0	54.0	54.0
0.6	42.7	45.5	47.4	48.3	48.3
1.2	31.1	37.8	39.2	39.2	39.2
1.8	30.2	34.9	39.2	39.6	39.6

TABLE A-5. PERCENT MIGRATION (TO JULY 6, 1977) OF YEARLING COHO SALMON RELEASED INTO A SMALL COASTAL STREAM FOLLOWING ACUTE AND CHRONIC EXPOSURE TO DINOSEB (RELEASED MAY 5, 1977).

Nominal concentration µg/L		Percent migration Days post release 1-5	6-10	11-20	21-30	31+
1. Acute exposures - 96 h						
Control	(115)[a]	60.0	63.5	66.1	66.1	66.1
10	(113)	53.1	55.7	55.7	56.6	57.5
20	(98)	62.2	66.3	67.3	68.4	68.4
40	(110)	47.3	51.8	54.5	54.5	54.5
60	(105)[b]	43.6	52.5	54.5	56.4	56.4
2. Chronic exposures - 285 h						
Control	(103)	47.6	52.4	54.5	54.4	54.4
Control	(100)	59.0	65.0	65.0	65.0	65.0
10	(104)	59.6	65.4	68.2	69.2	69.2
10	(105)	60.9	68.6	71.4	72.4	72.4
20	(100)	59.0	64.0	66.0	66.0	66.0
20	(103)	43.7	49.5	50.5	51.5	51.5
40	(109)	39.4	53.2	55.0	56.0	56.0
60	(88)[c],[d]	26.1	35.2	37.5	37.5	38.6
60	(109)[c]	35.8	49.5	56.9	56.9	56.9

[a] *Number of fish released in parenthesis*
[b] *24 h exposure*
[c] *48 h exposure*
[d] *Some mortality occurred in the exposure tanks prior to marking and release.*

TABLE A-6. CONCENTRATION OF DINOSEB IN VARIOUS TISSUES OF YEARLING COHO SALMON FOLLOWING EXPOSURE TO SUBLETHAL AND LETHAL CONCENTRATIONS OF DINOSEB[a/].

Exposure concentration	Sample	Concentration of dinoseb (µg/L)						
		skin	muscle	gill	spleen	gall bladder	liver	kidney
Control[b/]	a	<0.01	<0.01	<0.09	<0.28	<0.11	<0.05	<0.04
	b	<0.03	<0.09	<0.15			<0.29	<0.09
20 µg/L (384 h)	a	<0.02	<0.01	0.09 (0.06)	1.4 (0.3)	<0.15	0.40 (0.06	0.37 (0.05)
	b	<0.03	<0.01	0.07 (0.03)			0.15 (0.03)	0.11 (0.03)
60 µg/L (144 h)	a	<0.02	<0.01	<0.08	0.61 (0.38)	0.77 (0.23)	<0.07	<0.04
	b	<0.03	<0.01	<0.09			<0.08	<0.11

[a/] *On basis of wet weight of tissue examined; to obtain an adequate tissue sample generally 3-5 fish were combined.*

[b/] *Values given as less than (<) a number are below the detection limits; numbers in parentheses below a number indicate the detection limit.*

TABLE A-7. EFFECT OF DIQUAT EXPOSURE ON AVERAGE LENGTH, WEIGHT, AND CONDITION FACTOR.

Date of sample	Days of exposure	Tank No.	Nominal concentration (mg/L)	Fork length cm ± SE	Weight g ± SE	Condition factor KFL ± SE
May 10, 1977	0[a]	1	Control	16.2 ± 0.16	42.7 ± 1.35	0.994 ± 0.008
	0	2	Control	16.8 ± 0.27	47.1 ± 2.15	0.980 ± 0.011
	0	7	0.5	16.8 ± 0.31	48.2 ± 2.84	0.992 ± 0.009
	0	8	0.5	17.3 ± 0.38	54.2 ± 3.51	1.009 ± 0.009
	0	3	1.0	16.4 ± 0.24	44.3 ± 1.91	0.988 ± 0.008
	0	4	1.0	16.9 ± 0.27	48.7 ± 2.18	0.985 ± 0.011
	0	5	2.0	16.4 ± 0.25	46.3 ± 2.01	1.026 ± 0.009
	0	6	2.0	16.9 ± 0.29	50.3 ± 2.59	1.019 ± 0.011
	0	9	3.0	16.6 ± 0.25	46.9 ± 2.25	1.001 ± 0.009
	0	10	3.0	16.9 ± 0.22	49.6 ± 1.98	1.005 ± 0.008
May 26, 1977	0[b]	1	Control	16.8 ± 0.20	47.3 ± 1.67	0.986 ± 0.008
	0	2	Control	16.5 ± 0.20	44.8 ± 1.54	0.984 ± 0.009
	12	7	0.5	17.5 ± 0.28	53.6 ± 2.90	0.977 ± 0.007
	12	8	0.5	16.9 ± 0.26	49.2 ± 2.28	0.990 ± 0.009
	12	3	1.0	16.5 ± 0.16	45.3 ± 1.59	0.987 ± 0.013
	12	4	1.0	17.0 ± 0.21	48.9 ± 1.88	0.972 ± 0.013
	12	5	2.0	17.3 ± 0.25	52.9 ± 2.63	0.994 ± 0.010
	12	6	2.0	17.1 ± 0.21	50.7 ± 1.97	1.003 ± 0.015
	12	9	3.0	16.7 ± 0.23	46.7 ± 2.11	0.981 ± 0.008
	12	10	3.0	17.1 ± 0.22	49.6 ± 1.77	0.979 ± 0.009

[a] Sample size 30
[b] Sample size 40

TABLE A-8. PERCENT MIGRATION (TO JULY 6,1977) OF YEARLING COHO SALMON RELEASED INTO A SMALL COASTAL STREAM FOLLOWING ACUTE AND CHRONIC EXPOSURE TO DIQUAT (RELEASED MAY 26, 1977).

Nominal concentration mg/L	Percent migration Days post release 1-5	6-10	11-20	21-30	31+
1. Acute exposure - 96 h					
Control	66.0	71.8	72.8	72.8	72.8
0.5	47.6	58.1	58.1	58.1	58.1[a]
1.0	54.5	60.4	62.4	62.4	62.4
2.0	38.8	46.9	47.9	47.9	47.9[a]
3.0	41.0	53.0	53.0	53.0	53.0[a]
2. Chronic exposures - 285 h					
Control	55.2	62.3	63.4	63.4	63.4
0.5	43.2	53.9	55.5	55.5	55.5
1.0	38.7	47.5	48.1	48.1	48.1[a]
2.0	25.6	41.7	42.2	42.2	42.2[a]
3.0	16.9	26.4	26.4	26.4	26.4[a]

[a] *Migration rates significantly lower than controls (P = 0.05).*